

Matelea radiata* Correll (Apocynaceae, Asclepiadoideae) Rediscovered in South Texas*Richard E. Rintz**3455 County Road 4080, Salem, Missouri 65560, U.S.A.
rasasiang@hotmail.com**ABSTRACT**

A single plant of *Matelea radiata* Correll was found in Starr Co., Texas in Aug, 2005. Photos were taken and flowers placed in liquid for analysis. This is our first good look at this rare plant. Published on-line www.phytologia.org *Phytologia* 96(1): 1-6 (Jan. 8, 2014). ISSN 030319430.

KEY WORDS: *Matelea radiata*, Starr Co., TX, rediscovery.

INTRODUCTION

On Jun 24, 1909 the American botanist and author, Frederick Lewis Lewton, collected a specimen from a perennial vine near Falfurrias in what is now Brooks Co. Texas. The specimen (*F. L. Lewton* 828) made its way into the herbarium at the National Arboretum in Washington, D.C. and became NA no. 271771. However, it was not identified until 1965 when Donovan Stewart Correll described it as the new species, *Matelea radiata*. A rare plant, it may not have been seen since Lewton first collected it nearly a century ago. A specimen (*Runyon* 2832 at TEX) collected by Robert Runyun on Jul 13, 1941 is disputed to be this species, as is *Wood* 762 at TEX, collected by Archie Wood on Apr 4, 1966. Both are from Starr Co. Correll did not cite the Runyon specimen in his paper. The problem apparently arises because another species within the same range, *M. sagittifolia* (Gray) Woodson, is nearly identical in habit, though it has a shorter and entirely cup-shaped corona. The corona as described by Correll was not well understood. This paper should correct that problem.

OBSERVATIONS & CONCLUSIONS

While searching for *M. sagittifolia* in Aug, 2005 in Starr Co., TX, I came across a perennial vine twining over 2 meters up into a tree near Falcon Village on the Rio Grande River. Because of its habit with small sagittate leaves, I took it to be the species I was in search of. It had only a few flowers, but I was able to photograph several and put 2 in alcohol for later analysis under the microscope. It wasn't until 2013 when I saw photos of *M. sagittifolia* taken by W. R. Carr on the internet that I realized that I had something different.

The plant I found is a good match for the one described by Correll. It is a slender, twining vine with small sagittate leaves, and has the flowers mostly solitary in the leaf axils on a very short peduncle. But most notably it has *long, oblong-quadrate coronal appendages*, that are '*broadly concave-emarginate at the truncate apex, with a flange-like keel arising at the base on the ventral surface and often extending to well above the middle.*' Correll described the corolla as 'spreading-radiate,' but he was describing pressed flowers. On the living flowers the corolla does not open so far as to be radiate, but is more campanulate in general form. There

is a fine drawing of the plant in RARE PLANTS OF TEXAS by Linny Heagy made from the holotype and shows the flowers and the coronas pressed flat and radiate. On living flowers the corona is an erect & deeply 5-cleft cylinder, very distinctive and unlike any other *Matelea* native to the U.S. A feature that Correll missed is the conical stigma. On most other U.S. species of *Matelea* the stigmas are flat-concave or slightly convex. The stigma on *M. cynanchoides* is closest to this one, being convex. The pollinarium is typical of the genus in the U.S.

ACKNOWLEDGMENTS

I extend my gratitude to Dana M. Price, Chris Best, and to Tom & Elena Patterson, without whose assistance I would not have been in South Texas to find the plant; and to W. R. Carr whose photos of *M. sagittifolia* on the LBJ Wildflower site called my attention to my error in identification.

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Figure 1. Flower of *Matelea radiata* with two petals removed to show the erect-columnar corona. Insert shows the pollinarium (about 1 mm long). Photo by the author from a flower in liquid. The entire flower is about 1 cm long.

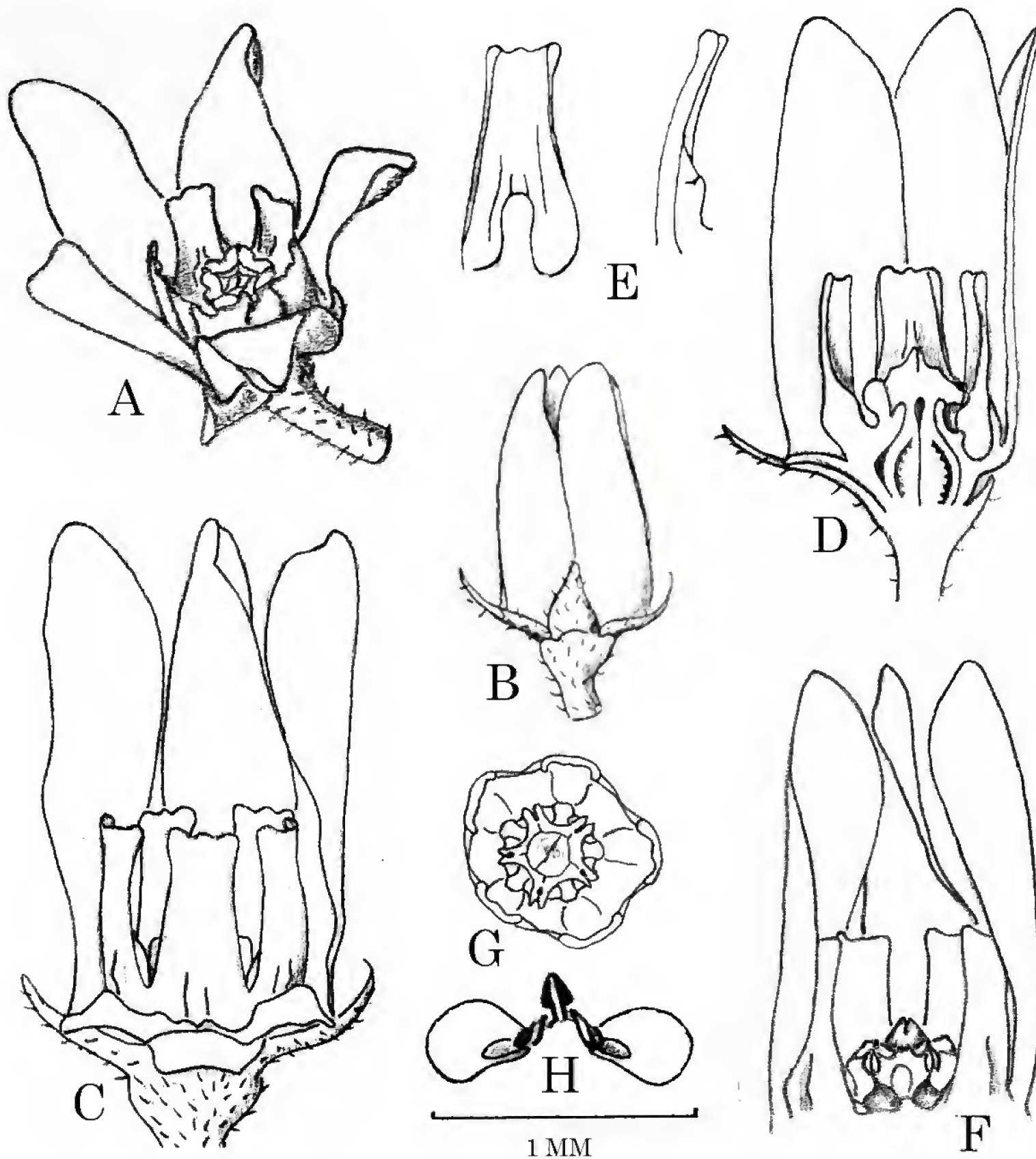


Figure 2. *Matelea radiata* Correll. A, open flower; B, bud; C, flower with two petals and a sepal removed to show the erect-columnar corona; D, flower in radial section; E, coronal segments in ventral & lateral views; F, flower cut away to show the gynostegium with conical stigma; G, gynostegium from above; H, pollinarium. From a flower in alcohol collected by the author at the site.



Figure 3. Photo of a living plant with one open flower and one closed flower. Taken by the author at the site.



Figure 4. Photo of a fruit taken by the author at the site.

Taxonomic overview of *Eustoma* (Gentianaceae)

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ABSTRACT

The genus *Eustoma* is treated as having two partially sympatric species: a widespread, small-flowered, *E. exaltatum*; and a more restricted, large-flowered *E. russellianum*. In spite of their extensive overlap, hybrids between the two are only rarely detected in natural populations, or inferred but rarely from among numerous sheets on file at TEX. The Mexican species, *E. barkleyi* Shinnars, is treated as but a form of *E. exaltatum* [**f. barkleyi** (Shinnars) B.L. Turner, **forma nov.** Maps showing distributions of the several taxa are provided. Published on-line www.phytologia.org *Phytologia* 96(1): 7-11 (Jan. 8, 2014). ISSN 030319430

KEY WORDS: Gentianaceae, *Eustoma*, USA, Mexico

EUSTOMA Salisb.

Annual to short-lived perennial herbs to 80 cm high. **Leaves** simple, opposite, sessile, often clasping, glabrous, more or less glaucous, ovate to linear-lanceolate. **Flowers** showy, borne on long pedicels, terminal and solitary or paniculate. **Calyx** deeply cleft, the lobes attenuate, erect, glabrous. **Corolla** deeply campanulate, the lobes large, erect, glabrous, purple to lavender, pink to white. **Stamens** 5 or 6, inserted on the corolla throat; anthers versatile, linear, yellow. **Styles** erect, slender, glabrous; stigmas broadly 2-lobed, their upper surfaces densely and minutely glandular-pubescent. **Capsules** ellipsoid, 2-valved, glabrous. Seeds minute, numerous, reticulate, more or less globoid. **Base chromosome number**, $x = 18$.

Eustoma, as treated here, is a genus of only two species, in spite of the considerable synonymy listed for each. It is typified by ***E. exaltatum*** Salisbury, *Paradisus Londinensis*, t. 34 (1806), as noted by Shinnars (1957).

Key to species

1. Corolla lobes mostly 2.5-5.0 cm long, 20-30 mm wide; calyx lobes mostly 1.5-2.5 cm long ***E. russellianum***
1. Corolla lobes mostly 1.5-2.5 (3.0) cm long, 5-15 mm wide; calyx lobes mostly 1.0-1.5 cm long... (2)
2. Leaves mostly lanceolate, 2-6 mm wide, 5-12 times as long as wide; northeastern Mexico..... ***E. exaltatum* f. *barkleyi***
2. Leaves mostly ovate to ovate-lanceolate, 6-15 mm wide, 2-5 times as long as wide; widespread..... ***E. exaltatum***

EUSTOMA EXALTATUM (L.) Salisb. ex G. Don, Gen. Hist. Dichlam. Plants 4: 211. 1837.

Arenberia glauca Mart. & Gal.

Chlora exaltata (L.) Griesb.

Dupratzia scoparia Raf.

Erythraea plumieri H.B.K.

Eustoma chironioides (Benth.) Griesb. in DC.
Eustoma exaltatum f. *albiflorum* Benke
Eustoma exaltatum var. *albiflorum* Cham. & Schlecht.
Eustoma selenifolium Salisb.
Lisianthus exaltatus (L.) Lam.
Lisianthus glaucifolius Jacq.
Urananthus glaucifolius (Jacq.) Benth.

This species, and that which follows, is well described by numerous workers in many floras (e.g. Correll & Johnston 1970; Munz, 1965; etc.), and I see little need to add or subtract from such parameters. Indeed, the above generic description should suffice for both **E. exaltatum** and **E. russellianum**, the key to species gives the only characters that seem to differentiate between the two. Indeed, in the Illustrated Flora of North Central Texas (Shinners and Mahler, 1999), the two species are combined, but with reservation, awaiting the definitive work of yet others. After study of the two taxa, both in the field and in herbaria, I conclude that specific status for both is warranted.

According to a USDA website (ARS: 1995. Germplasm Resources Information Network): **E. russellianum** is native to the southern United States south to Mexico; while **E. exaltatum** is native to the southern United States, Mexico, Central America, and the West Indies. “The two species may represent different ecotypes of the same species, for both species are inter-fertile and produce fertile progeny.” In cultivation, **E. russellianum** is said to be a biennial, and first produces a rosette which bolts into a single flowering stem after cold treatment, then dies after branching. In contrast, **E. exaltatum** grows more like a perennial and produces additional shoots each season. Both species have purple flowers that are funnel-shaped to campanulate, but the corolla lobes of **E. exaltatum** range up to 2.5 cm in length, whereas those of **E. russellianum** are 5 to 6 cm in length; most of the material in cultivation is said to belong to the latter species. According to the relative few chromosome reports available, both species are diploid with $n = 32$ chromosomes.

The biological assessment of the ARS seems sound, except that I would recognize the “ecotypes” as species, as noted below.

It should be emphasized that the two taxa are broadly sympatric. In spite of this, they are only rarely found growing together, presumably because of edaphic factors (heavy saline or clay soils, mostly occurring in water-logged soils or seeps in **E. exaltatum**; sandy-clay or calcareous soils along stream sides in **E. russellianum**); indeed, putative hybrids between these are apparently rare, strongly suggesting that they are deserving of specific status, instead of infraspecific status for **E. russellianum**, as bestowed by Kartesz (1999).

Richardson and King (2011) provided an excellent account of the species as it occurs in southern Texas, including a colored photograph from Willacy Co. (pers. comm.).

Shinners (1957) noted that white-flowered forms of the species occur, the earliest given varietal recognition by Chamisso and Schlechtendal in 1831, as noted in the above synonymy.

EUSTOMA EXALTATUM f. BARKLEYI (Standl. ex Shinners) B.L. Turner, **forma nov.**
 Based upon *Eustoma barkleyi* Standl. ex Shinners, Southwestern Naturalist 2: 39. 1957.
 Type: **MEXICO. COAHUILA:** “inside hot springs enclosure,” Ojo de Caliente [33 mi SW of Monterrey], *Hernandez et al.* 16M548 (Holotype: TEX!).

Shinners, in his original description, aptly noted:

The open branching and very narrow leaves make this [taxon] very distinct in the genus whose species are separated only by vegetative and size differences. It is allied to *E. exaltatum* in its small flowers.

Shinners further noted that **forma barkleyi** is apparently restricted to northeastern Mexico and that it appears to intergrade with **E. exaltatum**, this “presumptive evidence” that natural hybridization between the two occurs. I toyed with the idea of accepting specific status for the taxon, noting that the two leaf forms may occur at the same site (e.g. Coahuila: 19 km SW of Cuatro Cienagas, *Chaing et al.* 7645 [TEX, both mounted on the same sheet], but the degree of sympatric intergradation between the two leaf forms and their lack of geomorphological integrity (cf. Fig. 1), has led me to reduce *E. barkleyi* to the status of forma w/o much ado. Apparently K.N. Gandhi of Harvard University took the same view, having annotated all such leaf forms at TEX as **E. exaltatum**.

Interestingly, a single collection from among the hypothetical hybrid populations from Cameron Co., Texas, discussed under **E. russellianum**, below, possesses the approximate leaf shape of **f. barkleyi** (*Runyon* 5098, TEX), which suggests that ancestral hybridization between the two species might have given rise to **f. barkleyi**.

Amongst the synonymy, above and below, it will be noted that numerous color-forms have been described for the two species, and I have observed additional leaf shapes among collections of **E. exaltatum** that I opted not to describe.

EUSTOMA RUSSELLIANUM (Hook.) G. Don ex Sweet, Hort. Brit. ed. 3: 473. 1839.

Bilamista grandiflora Raf.

Eustoma andrewsii (Hook.) G. Don ex Sweet

Eustoma exaltatum subsp. *russellianum* (Hook.) Kartesz

Eustoma gracile A. Gray

Eustoma grandiflorum (Raf.) Shinners

Eustoma grandiflorum **f. album** (Holz) Waterf.

Eustoma grandiflorum **f. bicolor** (Standl.) Shinners

Eustoma grandiflorum **f. fisheri** (Standl.) Shinners

Eustoma grandiflorum **f. flaviflorum** (Cockerell) Shinners

Eustoma grandiflorum **f. roseum** (Standl.) Shinners

Eustoma russellianum **f. bicolor** Standl.

Eustoma russellianum **f. fisheri** Standl.

Eustoma russellianum **f. flaviflorum** Cockerell

Eustoma russellianum **f. roseum** Standl.

Eustoma russellianum var. *flavum* A.M. Davis

Eustoma russellianum var. *gracile* A. Gray

Lisianthus russellianus Hook.

Urananthus russellianus (Hook.) Benth.

As to description of this species, and its geomorphological relationship with **E. exaltatum**, see above. Its distribution is shown in Fig. 3. It should be noted that I have not seen collections of **E. russellianum** from Mexico; most such reports by others have been based upon unusually large flowered specimens of, otherwise, typical **E. exaltatum**.

Enquist (1987) provides an excellent, colored, close up of the corolla of **E. russellianum**; note its contrast with the smaller flowered, **E. exaltatum** (Richardson & King 2011).

Many of the forms listed in the above synonymy are probably the result of occasional natural hybrids between *E. exaltatum* and *E. russellianum*, this exemplified by a number of such forms occurring in easternmost Texas (e.g., Montgomery Co.: Willis, *Fisher s.n.* (TEX), this also the county from which the Types of both **forma bicolor** and **f. roseum** were obtained). Especially noteworthy are a number of seemingly intermediates and hypothetical backcrosses between the two species in southernmost Texas (Cameron Co.), where the two species apparently occur together in heavy clay or sandy loam soils (16 sheets assembled by 9 or more collectors over the years 1923-1967, most of these identified as one or the other species, but at least one bearing the name of *E. russellianum* var. *flavum* A.M Davis, the holotype

(TEX) reportedly collected in a recent delta area of that county by its collector, the type not located by Shinnars, nor does it exist among the type specimens of that herbarium today.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. G. N. Gandhi of Harvard Univ. for providing the publication date of *E. russellianum*, thus preempting by several months or more that of *E. grandiflorum*, the latter used by my ex-mentor, Lloyd Shinnars. Thanks to Jana Kos for editorial assistance and to Matt Turner and Paul Waller for observations and collections from two large populations of *E. russellianum* in north central Texas. Distribution maps are largely based upon specimens on file at LL-TEX, SRSC, and various other reports in the literature, including the USDA website, that appeared reasonable to the present author.

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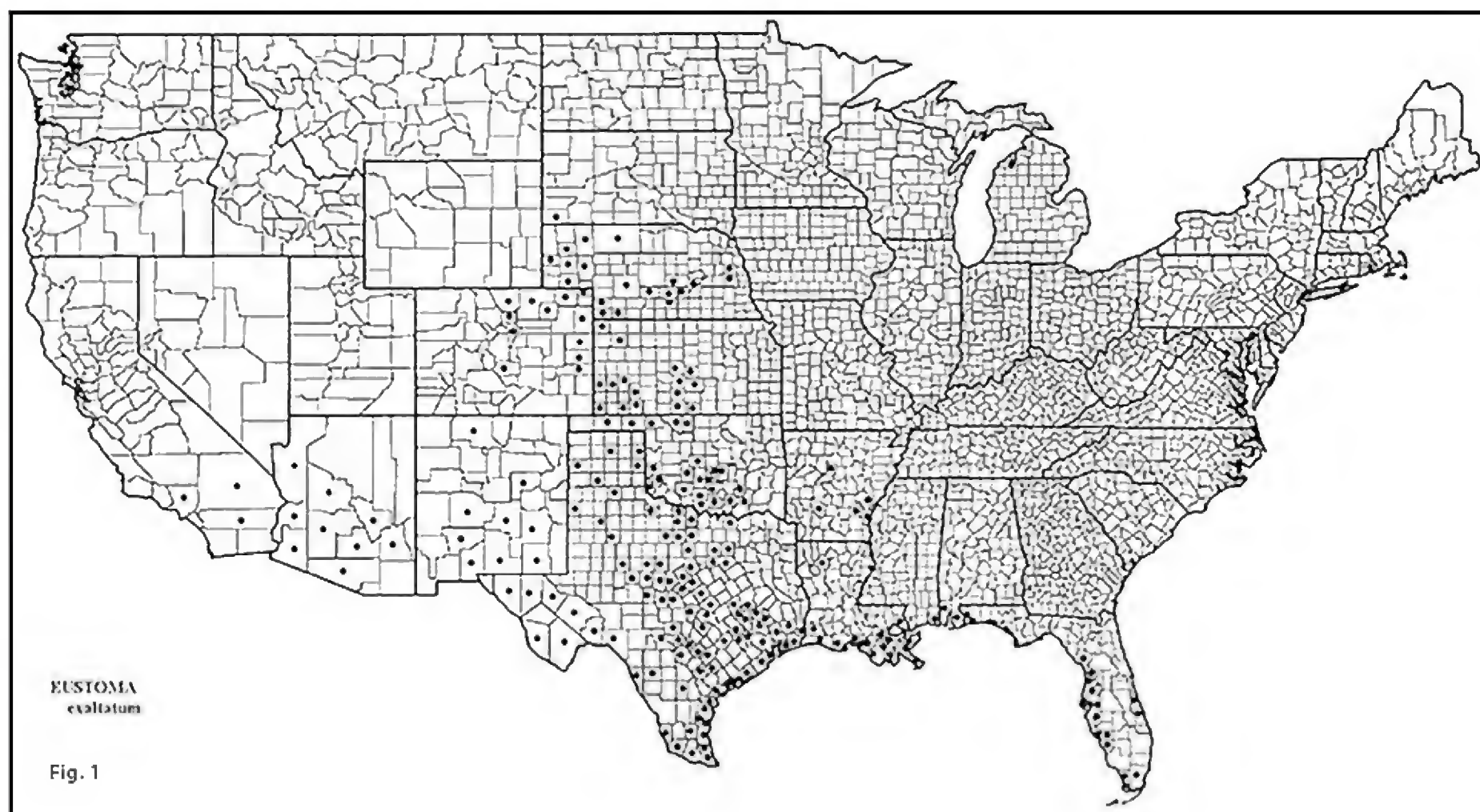


Fig. 1. Distribution of *Eustoma exaltatum* in the USA.

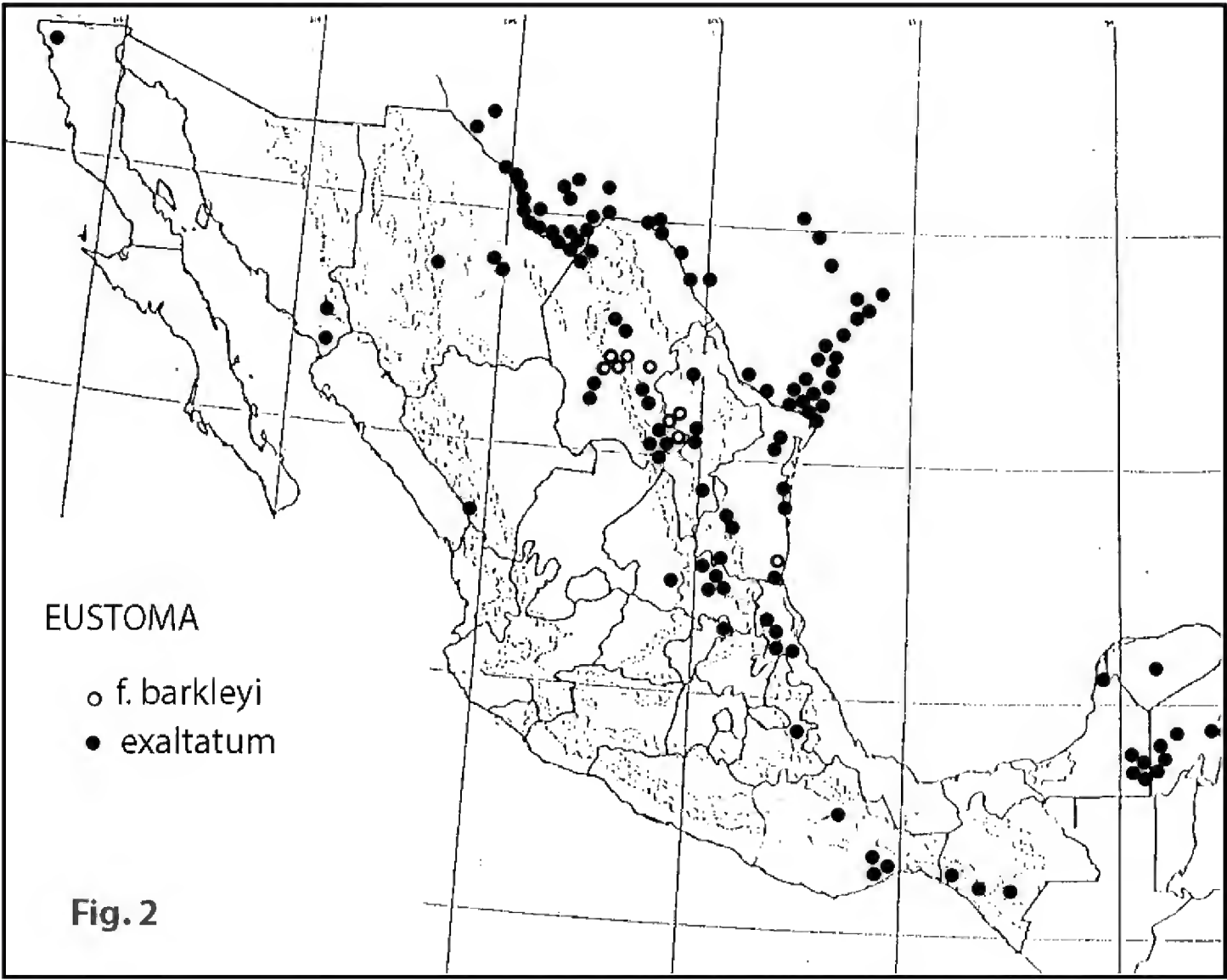


Fig. 2. Distribution of *Eustoma exaltatum* and *E. e. f. barkleyi* in Mexico.

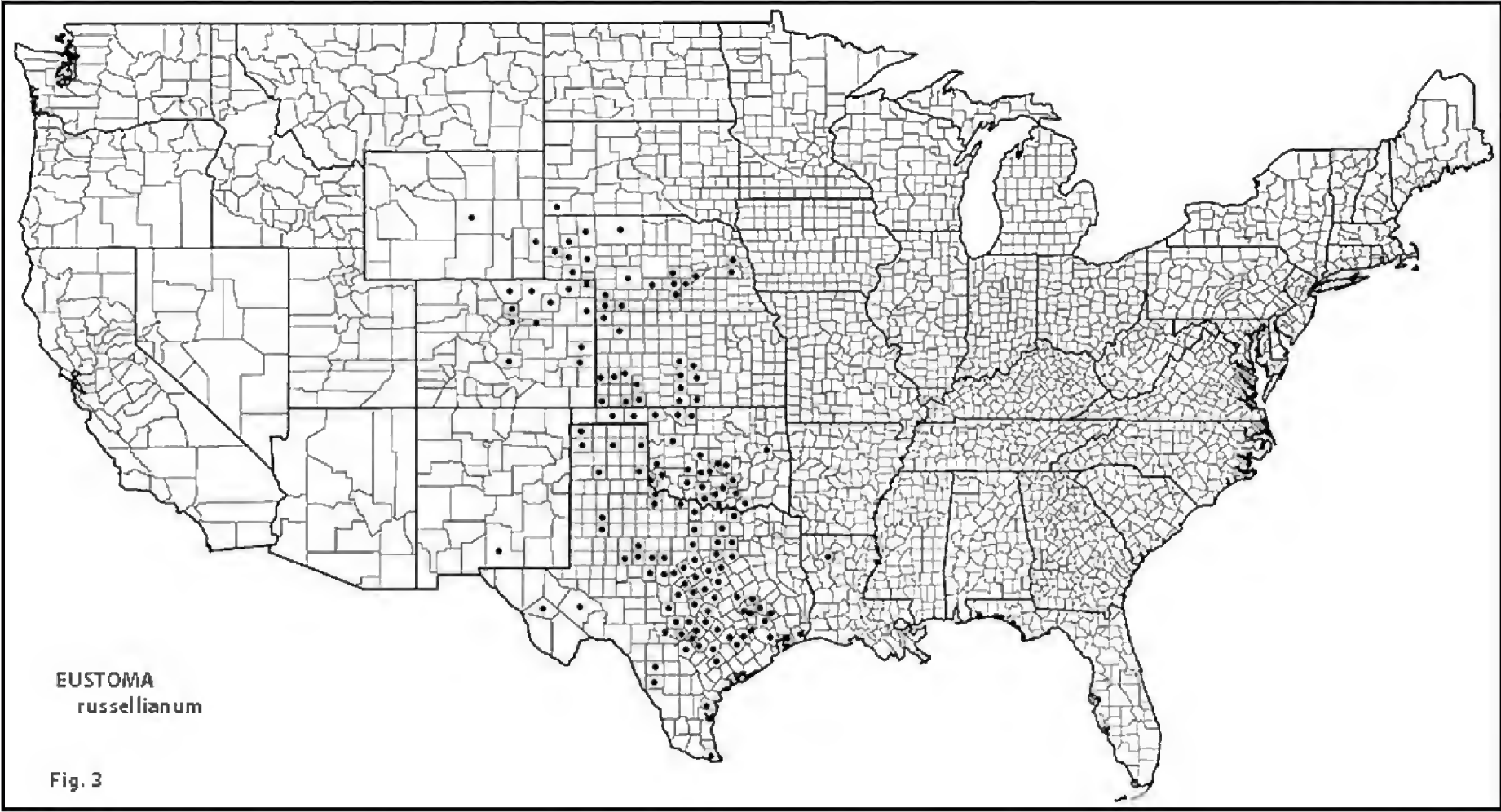


Fig. 3. Distribution of *E. russellianum*.

Taxonomy of the *Ageratina vernalis* (Asteraceae: Eupatorieae) complex

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ABSTRACT

A taxonomic re-study of the broadly distributed, highly variable, *Ageratina vernalis* complex is presented. From among its midst, two novelties are extracted: **A. jalpana** B.L. Turner, **sp. nov.** from Queretaro; and **A. zaragozana** B.L. Turner, **sp. nov.** from Nuevo Leon. A complete synonymy is presented, along with photographs of the types and distribution maps.

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KEY WORDS: Asteraceae, Eupatorieae, *Ageratina*, *A. vernalis*, Mexico, Guatemala

In my treatment of **Ageratina** for Mexico (Turner 1997), I provided an abbreviated account of **Ageratina vernalis**, including within this material that I now treat as two novelties that are described below.

AGERATINA VERNALIS (Vatke & Kurtz) King & H. Rob., Phytologia 19: 227. 1970.

Ageratina skutchii B.L. Rob.

Ageratina subcoriacea King & H. Rob.

Ageratina subinclusa (Klatt) King & H. Rob.

Ageratina subpinninervia (Klatt) King & H. Rob.

Eupatorium grandiflorum Andre

Eupatorium melanolepis Sch.-Bip. ex Klatt

Eupatorium monticola L. Williams

Eupatorium oxylepis Sch.-Bip. not *E. oxylepis* DC.

Eupatorium subinclusum Klatt

Eupatorium subpinninervium Sch.-Bip.

Eupatorium vernale Vatke & Kurtz

Dur, Nue, Tam, Hid, Ver, Pue, Oax, Cps and Guatemala, oak and pine-oak forests, mostly in rocky soils, 2000-3000 m; flowering: Oct-Jan. **Map 1**

Shrubs 2-4(6) m high. **Stems** (upper) rounded, 3-5 mm across, markedly pubescent with mostly upswept crinkly hairs, glabrate with age, the indument ca 1 mm high. **Leaves** (mid-stem) 10-20 cm long, 4-8 cm wide; petioles 3-5 cm long; blades broadly ovate to subcordate, sparsely pubescent above and below, mainly along the venation, the margins serrulate. **Capitulescence** a terminal cymose panicle of numerous heads, 5-10 cm high, 8- 15 cm wide, the ultimate peduncles 3-12 mm long. **Heads** 9-12 mm long, 6-8 mm wide; involucral bracts linear-lanceolate, 2-seriate, 3-6 mm long, their apices acute. **Receptacles** plane, glabrous, epaleate, ca 1.5 mm across. **Florets** ca 20 per head; corollas 6-7 mm long, white to purplish-pink, glabrous throughout; tube ca 1.5 mm long; throat ca 1.5 mm long, passing into the tube; lobes 5, ca 1 mm long, glabrous, or rarely with a few short hairs (e.g. Nuevo Leon: Cerro Viejo, *Hinton et al.* 23960, TEX). **Achenes** 2-4 mm long, black, sparingly pubescent along the ribs; pappus of 20-25 bristles, 4-6 mm long.

REPRESENTATIVE SPECIMENS EXAMINED: **GUATEMALA. DEPT. QUETZALTENANGO:** **Mpio. de Cantel**, "above Xecam on western slopes of Zunil Ridge (Sierra Chuatroj)," 1 Aug 2008, *Quedensley* 5247 (TEX); **Mpio., Quetzaltenango**, nw slopes of Volcan Santa Maria, 4 Jan 2008,

Quedensley 5200 (TEX); **Mpio. de Zunil**, nw slopes of Pico Zunil, 3 Jan 2008, *Quedensley 5193* (TEX); **DEPT. SAN MARCOS: Mpio. San Marcos**, 3 Jan 1992, *Prather 1067* (TEX).

MEXICO. CHIAPAS: Mpio. San Cristobal Las Casas, 16-20 km E of Chilil, 10 Nov 1976, *Breedlove 41401* (LL). **DURANGO: Mpio. Puerto Buenos Aires**, “8 km al O del Poblado La Ciudad,” 8 Nov 1978, *Garcia P. & Delgado S. 903* (TEX). **HIDALGO: Mpio. Jacala**, 20.2 mi NE of Jacala, 20 Dec 1991, *Soule 2920* (TEX). **NUEVO LEON: Mpio. Zaragoza**, Cuauhtemoc, 10 Nov 1994, *Hinton et al. 25076* (TEX). **OAXACA: Mpio. Mihuatlan**, “Siete Ocotes,” 21 Nov 1995, *Hinton et al. 26266* (TEX). **PUEBLA: Mpio. Teziutlan**, “2 km S of Teziutlan, 4 Dec 1983, *Barrie 770* (TEX). **TAMAULIPAS: Mpio. Guemes**, “El Chihue,” 10 Nov 1994, *Hinton et al. 25101* (TEX). **VERACRUZ: Mpio. Las Vigas**, EL Volcancillo, 30 Oct 1975, *J. Dorantes et al. 5098* (TEX).

In my treatment of *Ageratina* for Mexico (Turner 1997), this species will key directly to *A. vernalis*; my descriptive parameters of the latter in that account included material herein separated (below) as *A. zaragozana* and *A. jalpana*, both perennial herbs to 1 m tall, these not included in the above description.

Williams (1976), in his treatment of *Eupatorium* for Guatemala, positioned this species among his “EXCLUDED SPECIES,” largely because of the absence of a Type specimen, noting that the illustration which was published with the description of *Eupatorium vernale* showed the plant to possess “both penninerves and plinerves” leaf blades, “a condition not likely to occur in a single species.” My examination of the illustration shows the leaves to be fairly typical of what I take to be *A. vernalis*. A mounted specimen of the type from the Vatke herbarium, available on the web (Virtual Herbarium 2011) also matches well the taxon concerned. In addition, I have examined a photograph of a Berlin specimen (US) said to be the type of *Eupatorium subpenninervium*, a name I place in synonymy with *A. vernalis*, although B. Robinson (cf below) accepted both names in his treatment of *Eupatorium* for Mexico.

I follow H. Robinson (1990) in placing *A. skutchii* in synonymy with the present taxon, its pappus lobes presumably glabrous, but I have not examined type material.

In his treatment of *Eupatorium* (including *Ageratina*) for Mexico, Robinson (1961) accepted *E. vernale*, noting that it was “Described from cultivated material thought to have been of Mexican origin.” He distinguished his *E. vernale* from the closely related *E. subpinninervium* by the supposedly longer involucral bracts in the former, a character I find to be quite variable, and have therefore placed the latter in synonymy with *A. vernalis* (Turner 1997).

Since my treatment of *Ageratina* for Mexico (1997), I have studied anew the entire assemblage, and additional, collections of plants closely related to *A. vernalis* on file at LL-TEX, concluding that two additional species lie hidden within the complex, as follows:

AGERATINA jalpana B.L. Turner, sp. nov. Fig. 1

Perennial herbs to 1 m high. **Stems** (upper) rounded, ca 3 mm across, densely pubescent with crinkly trichomes, the indument 1-2 mm high. **Leaves** opposite, gradually reduced upwards, 5-10 cm long, 4-5 cm wide; petioles 1.5-3.0 cm long; blades ovate-cordate, having 7-9 pronounced ribs, their apices broadly obtuse to rounded, densely pubescent beneath like the stems, surfaces densely glandular-punctate, their margins evenly crenate. **Capitulescence**, a terminal cymose panicle, ca 5 cm high, 9 cm wide, the ultimate peduncles 1.0-1.5 cm long. **Heads** campanulate, numerous, ca 10 mm long, 4-6 mm wide. **Involucral bracts**, 5-6 mm long, linear-lanceolate, 2-seriate, glabrous or nearly so, their apices acute. **Receptacles** plane, epaleate, glabrous, ca 1.5 mm across. **Florets** ca 20/head; corollas glabrous, pink or rose-colored, 6-7 mm long; tubes ca 1.5 mm long; throat ca 4 mm long, grading into the tubes; lobes ca 1 mm long. **Achenes** (immature) ca 3 mm long, minutely ciliate, especially above; pappus of ca 25 ciliate bristles ca 3 mm long.

TYPE: **MEXICO. QUERETARO: Mpio. de Jalpan**, 3 km al SE de El Lobo, pine-oak forests, 1750 m, 7 Dec 1987, *Leonel Chavez 136* (Holotype: TEX).

According to label data, the plant is an herb 1 m high having rose-colored heads. It was reportedly abundant at the site of collection.

The species is named for the Municipio de Jalpan, whence the type; not to be confused with *A. jalpana* B.L. Turner from Zacatecas, this named for the city of Jalapa (Turner 1996).

The type was originally identified as “aff. *Eupatorium trinionum* McVaugh,” but the plant is much closer to *Ageratina vernalis*. The novelty is noteworthy for its very distinctive leaves, which are pinnately nervate with 7-9 pronounced ribs, markedly villous beneath and having apices that are broadly obtuse to rounded.

AGERATINA ZARAGOZANA B.L. Turner, *sp. nov.* **Fig. 2**

Perennial herbs to 40 cm high. **Stems** (upper) rounded, 1-2 mm across, minutely pubescent with upturned hairs, the vestiture ca 0.3 mm high. **Leaves** (upper) opposite, 4-8 cm long, 2-3 cm wide; petioles 1-2 cm long; blades acute basally, ovate, 3-nervate to weakly pinnately nervate, widest near the middle, or nearly so, grading into the petioles, glabrous on both surfaces, or nearly so, glandular punctate, the margins serrulate. **Capitulescence** a terminal cymose panicle, 2-3 cm high, 4-5 cm wide, the ultimate peduncles 5-10 mm long. **Heads** campanulate, 10-12 mm high, 5-8 mm wide bearing 10-19 florets. **Receptacles** plane, epaleate, glabrous, ca 2 mm across. **Involucral bracts** 2-3 seriate, 4-7 mm long, ca 1 mm wide, their apices acute, sparsely pubescent, and beset with amber globules. **Corollas** 6-8 mm long, glabrous; tubes ca 1.5 mm long, throats 4-5 mm long, passing into the tube, the lobes sparsely pubescent, ca 1 mm long. **Achenes** ca 3 mm long, sparsely ciliate; pappus of ca 25 ciliate bristles, 5-7 mm long.

TYPE: **MEXICO. NUEVO LEON: Mpio. General Zaragoza**, Cerro Viejo, “fir forest.” 2810 m, 20 Nov 2000, *Hinton et al.* 23977 (Holotype: TEX).

ADDITIONAL COLLECTION EXAMINED: **MEXICO. NUEVO LEON: Mpio. General Zaragoza**, 14.7 km SE of Zaragoza, southern slope of Cerro Viejo, “Mixed oak-pine-fir forest,” along small stream and along path from the dirt road to Cerro Viejo,” 2500-2520 m, 1 Nov 1999, *Yahara et al.* 1834 (TEX).

This novelty will not key to any meaningful name in my Mexican treatment of *Ageratina*, but I reckon it to belong to the *A. vernalis* complex, largely based upon head and floral features, although the leaves are amply different from that species, as will be noted in Fig. 2. It occurs in close proximity to *A. vernalis* on Cerro Viejo, as indicated in Map 1; indeed, it is possible that the occasional hybrid between these might occur, to judge from the abnormal Hinton collection called to the fore in the above account of the latter, the plant possessing very small leaves and occasional hairs on its corolla lobes.

The species is named for the Municipio Gen. Zaragoza, from whence the type.

Key to taxa within the *Ageratina vernalis* complex:

1. Shrubs 2-4 m tall; larger leaves 10-20 cm long.....**A. vernalis**
1. Herbs 0.3-1.0 tall; larger leaves 4-10 cm long...**(2)**
2. Leaf blades densely pubescent beneath, not tapering into the petioles;
heads bearing ca 20 florets; pappus bristles ca 3 mm long; Que.....**A. jalpana**
2. Leaf blades glabrous or nearly so, tapering into the petioles;
heads bearing 10-19 florets; pappus bristles 5-7 mm long; Nue.....**A. zaragozana**

ACKNOWLEDGEMENTS

I am grateful to Sultan Quedensley for information relating to his numerous Guatemalan collections of **A. vernalis**, and to George Hinton for habitat data relating to **A. zaragozana**. Jana Kos, my editorial assistant, provided helpful suggestions.

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Fig. 1. *Ageratina jalpana* (Holotype: TEX).



Fig. 2. *Ageratina zaragozana* (Holotype: TEX).

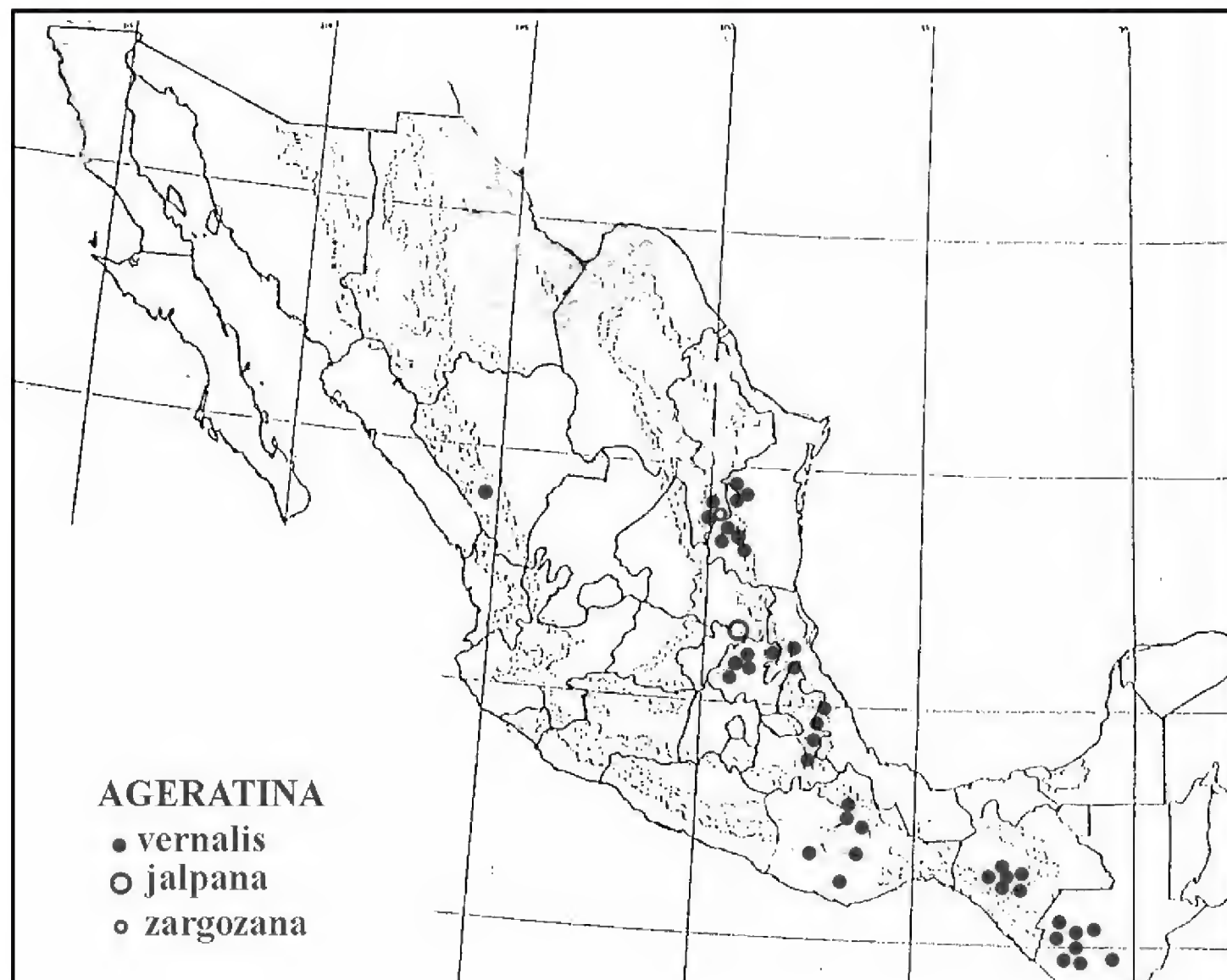


Fig. 3. Distribution of the *Ageratina vernalis* complex.

Taxonomy of *Juniperus* in Iran: DNA sequences of nrDNA plus three cpDNAs reveal *Juniperus polycarpus* var. *turcomanica* and *J. seravschanica* in southern Iran

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ABSTRACT

Sequence data from four gene regions (nrDNA, petN-psbM, trnD-trnT, trnS-trnG, 3,708 bp) showed strong support for the plants from Fasa in SW Iran being *J. p.* var. *turcomanica*. The samples from nearby south central Iran (Khabr) are part of a clade with *J. seravschanica*. Nine samples from Kuhbanan are in a clade with *J. seravschanica*, but 2 samples are in a clade with *J. p.* var. *turcomanica*. A minimum spanning network revealed that the Kuhbanan trees are genetically diverse and differentiated from more typical *J. seravschanica*. Nearly all samples from Kuhbanan were polymorphic in their nrDNA (ITS) chromatograms, implying either hybridization or incomplete lineage sorting. Additional research utilizing other nuclear genes (cf. ABI3, 4CL, etc.) will aid in understanding this complex. Published on-line www.phytologia.org *Phytologia* 96(1): 19-25 (Jan. 8, 2014). ISSN 030319430

KEY WORDS: *Juniperus polycarpus* var. *polycarpus*, *J. p.* var. *turcomanica*, *J. seravschanica*, *J. excelsa*, Cupressaceae, Iran, nrDNA, petN-psbM, trnD-trnT, trnS-trnG.

Previously we reported (Adams and Hojjati, 2012) that *Juniperus* from Iran has a complex mixture of taxa in southern Iran (Fig. 1); *J. polycarpus* var. *polycarpus* from Hashtjin (H), Lushan (L), and Qushchi, Q) were confirmed, as was *J. p.* var. *turcomanica* from Baladae (BL), Bajgiran (Bj) and Shahmirzad (Sh) in northern Iran. In southern Iran, the two plants from Fasa (F) were found to be *J. p.* var. *turcomanica* (Fig. 2). Two samples from Khabr were separated by 9 SNPs

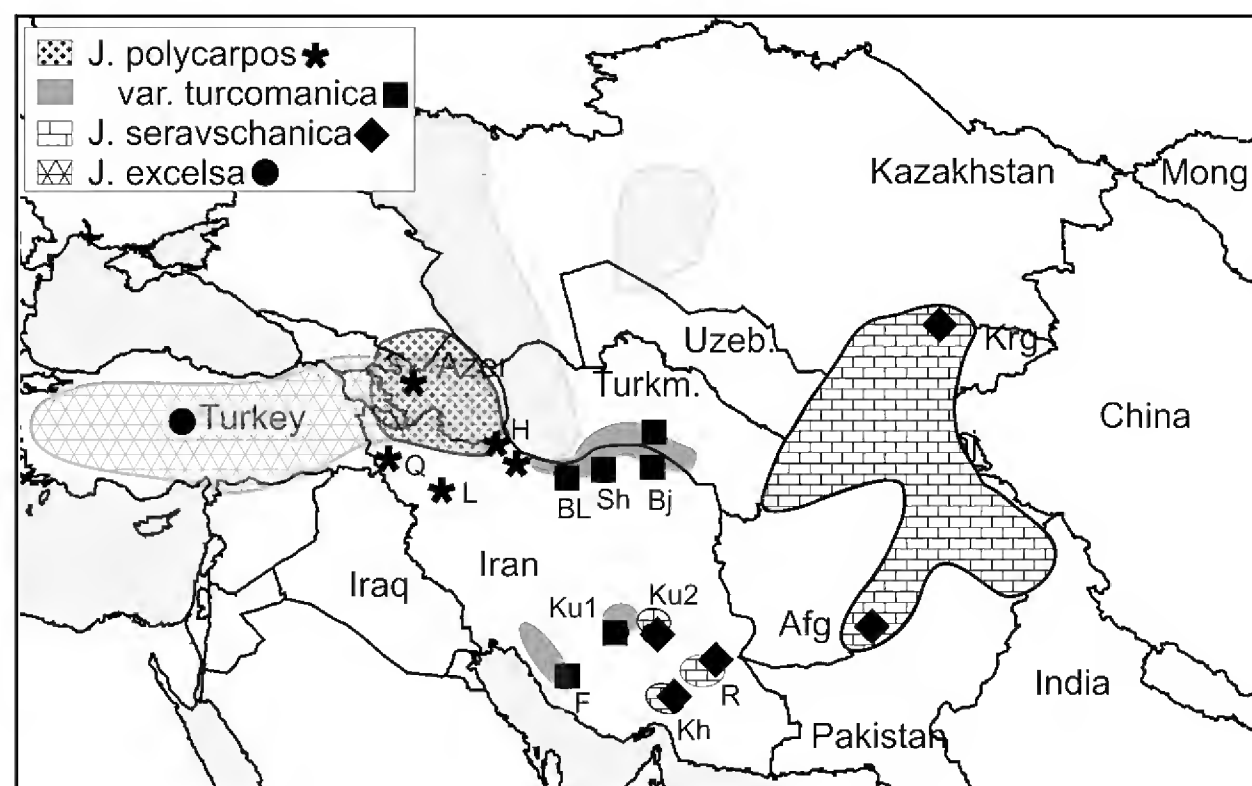


Figure 1. Dist. of *J. excelsa* (Greece not shown), *J. polycarpus*, *J. seravschanica*, and *J. p.* var. *turcomanica* (from Adams and Hojjati, 2012). Symbols indicate the popns. sampled for each taxon.

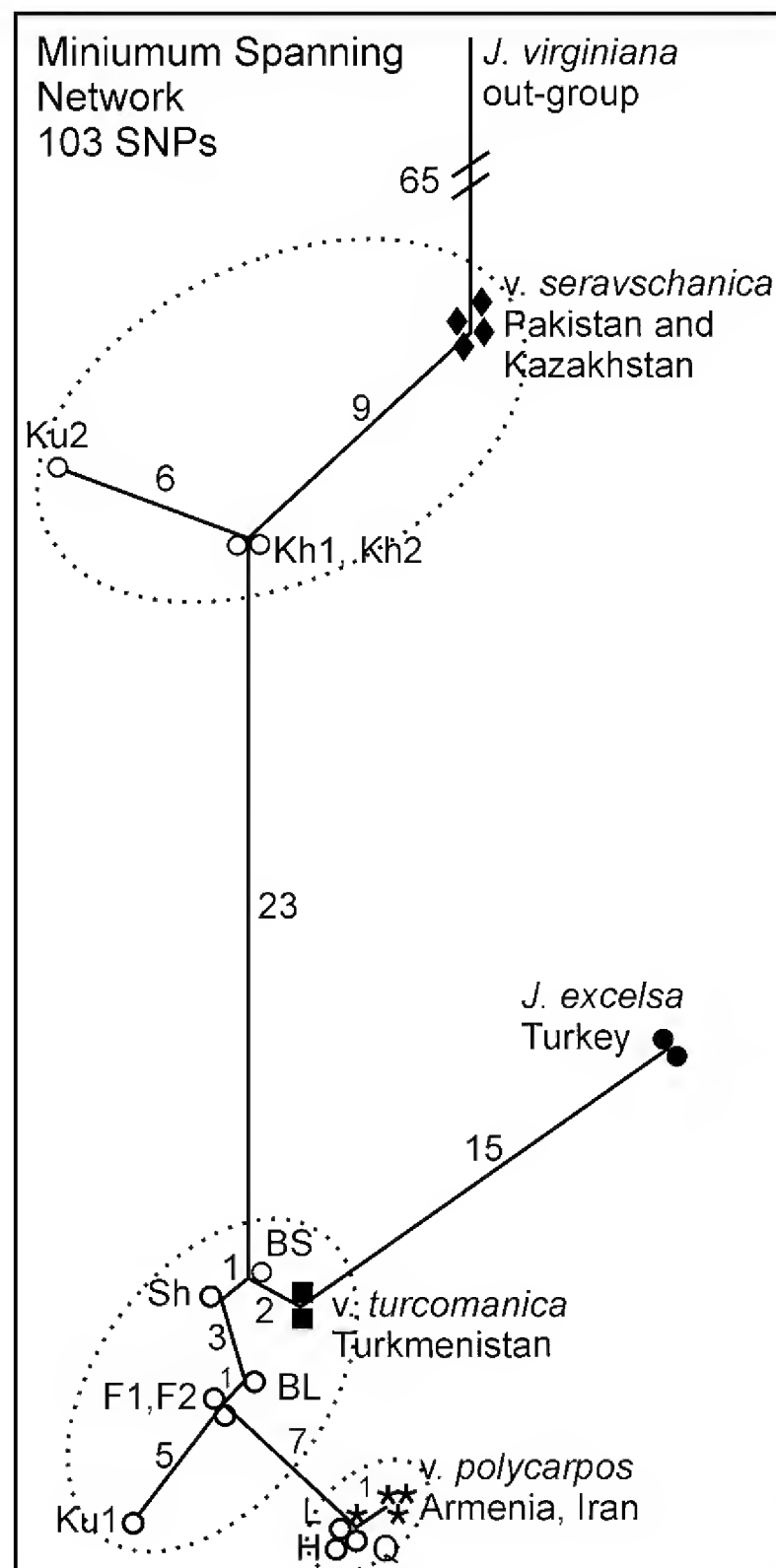


Fig. 2. Minimum spanning network. Numbers on lines are the number of SNPs for that link (from Adams and Hojjati, 2012).

(substitutions + indels) from *J. seravschanica* (Pakistan and Kazakhstan, Fig. 2). One sample from Kuhbanan (Ku1) was found to be *J. p. var. turcomanica* and the other (Ku2) was like *J. seravschanica* (Fig. 2). The Ku2 sample differed by 6 SNPs from Kh1 and Kh2, Suggesting that another infraspecific taxon of *J. seravschanica* might be present southern Iran.

To gather additional data, more samples were gathered from southern Iran and the leaf volatile oils analyzed (Adams and Hojjati, 2013).

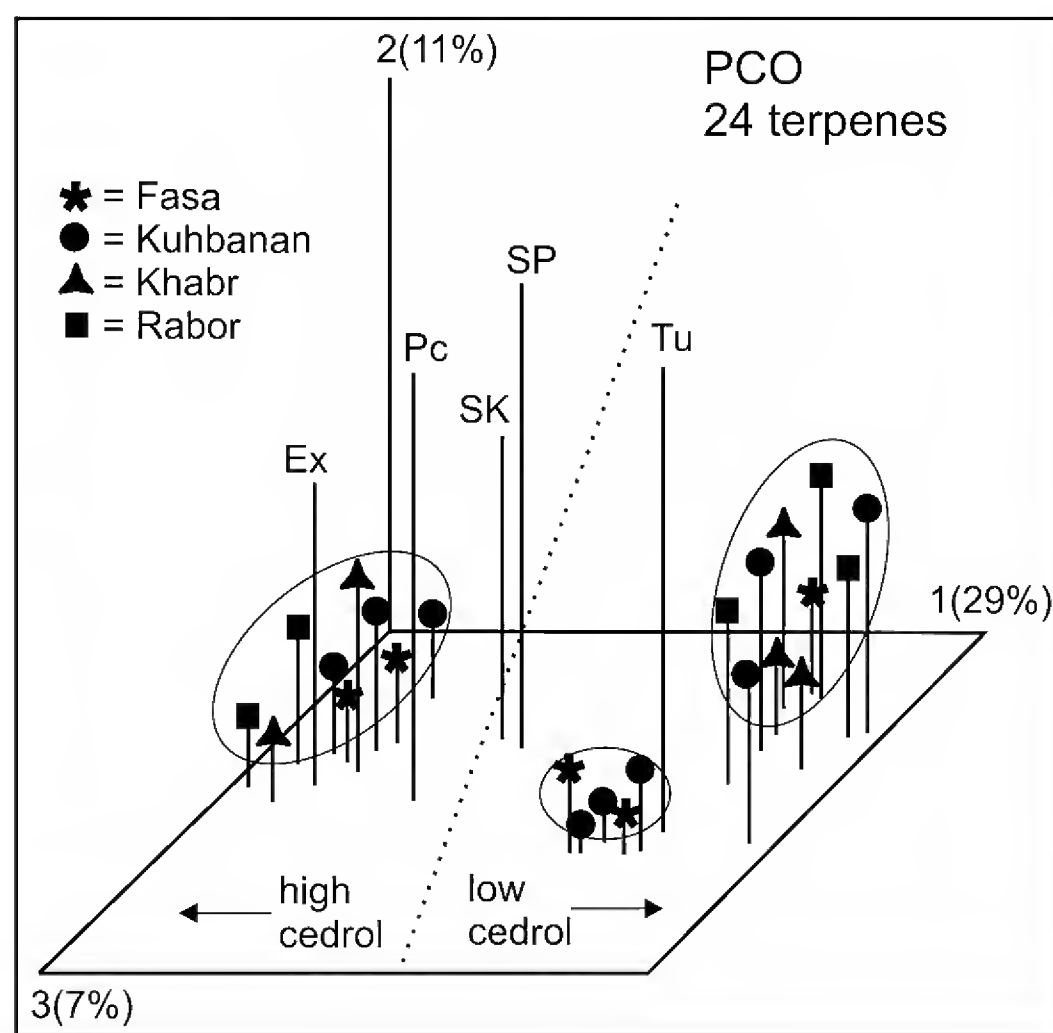


Fig. 3. PCO based on 24 terpenes of junipers of southern Iran. Ex= *J. excelsa*, Pc= *J. polycarpus*, SP, SK= *J. seravschanica*, from Pakistan and Kazakhstan (from Adams and Hojjati, 2013).

However, the oils were dominated by two chemotypes: high cedrol (wood oil sesquiterpenes) and low cedrol (leaf oil terpenoids). These chemotypes appeared to group irrespective of locations (Fig. 3). Interestingly, none of the oils from recognized species was closely grouped with the oils of junipers from southern Iran except *J. excelsa* (Fig.3).

Because the original DNA study (Adams and Hojjati, 2012) utilized only 2 samples from each of three southern Iran populations, additional samples were analyzed. The purpose of the present study was to utilize DNA sequence data from nrDNA, petN-psbM, trnD-trnT, trnS-trnG regions to analyze additional samples of *Juniperus* from southern Iran so as to better discern the taxonomic patterns in that region.

MATERIALS AND METHODS

In order to address variation in Iranian junipers, samples were selected from the same populations (Fig. 1) examined by Hojjati et al. (2009). DNA was extracted from plant materials from the following Hojjati populations (Popn. # and symbols are compatible with Hojjati et al., 2009):

Popn 1 L1, Lushan: *Adams 12789-12791*, 36° 40' 27"N, 49° 38' 49.5" E, Oct, 2006, Popn 2:L2, Lushan *Adams 12792-94* (3), 36° 40' 50" N, 49° 42' 24" E, Oct. 2006, Popn 3: H, Hashtjin *Adams 12795-12797*, 37° 26' 59" N, 48° 24' 13" E, Oct., 2006, Popn 4: Q, Qushchi *Adams 12798*, 38° 01' 20.3" N, 44° 57' 45.5" E, Oct., 2006, Popn 5: Sh, Shahmirzad *Adams 12799-12801*, 35° 50' 55" N, 53° 26' 24.2" E, Nov, 2006, Popn 6: Bj, Bajgiran *Adams 12802-12804*, 37° 25' 9.8" N, 58° 32' 0.2" E, Nov, 2006, Popn 7: G, Golestan *Adams 12805-12807*, 37° 19' 46.3" N, 56° 02' 34.2" E, Nov, 2006, Popn 8: BL, Baladae *Adams 12808*, 36° 14' 34.4" N, 51° 50' 20.4" E, Oct, 2006, Popn 9: F, Fasa *Adams 12809-12811*; Dec, 2006, *Adams 13754-13758*, Oct. 2012, 29° 09' 57.8" N, 53° 40' 7.8" E, Popn 10: Ku, Kuhbanan *Adams 12812-12814*, Jan, 2007, *Adams 13759-13767*, Oct. 2012, 31° 28' 21.5" N, 55° 52' 58.9" E, Popn 11: Kh, Khabr *Adams 12815-12817*, Jan, 2007, *Adams 13768-13772*, Oct. 2012, 28° 51' 8.4" N, 56° 22' 51.7" E, R, Rabor *Adams 13773-13777*, Oct. 2012, 28° 49' 06.7" N; 56° 21' 21.7" W. elev. 2086 m.

Authentic, typical taxonomically identifiable reference taxa, were included from *J. excelsa*, n of Eskisehir, Turkey, *Adams 9433-9435*, *J. polycarpus* var. *polycarpus*, Lake Sevan, Armenia, *Adams 8761-8763*, *J. p.* var. *turcomanica*, Kopet Mtns., Turkmenistan, *Adams 8757-8760*, *J. seravschanica*, Quetta, Pakistan, *Adams 8483-8485*, Dzhabagly, Kazakhstan, *Adams 8224-8226*; Elburz Mtns., Iran, *Shanjani s. n.*, (see Adams and Shanjani, 2011) [= *Adams 12603, 12604*]. Voucher specimens are deposited at Baylor University (BAYLU).

DNA Analysis - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams and Schwarzbach (2013) for the ITS, petN-psbM, trn D-trnT and trnS-trnG primers utilized. The PCR was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/>). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Sequence data sets were analyzed using Geneious v. R6-4 (Biomatters). Available from <http://www.geneious.com/>, and the MAFFT alignment program. Further analyses utilized the Bayesian tree analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

The Bayesian tree based on 3,708 bp shows (Fig. 4) strong support for the distinct nature of *J. excelsa*, *J. polycarpus*, *J. p.* var. *turcomanica*, and *J. seravschanica*. as found in other studies (Adams,

Morris and Schwarzbach, 2008; Adams, 2011; Adams and Schwarzbach, 2013). The samples from north- western Iran (I1, I2, L, H, Q) are *J. polycarpus* (Fig. 4). Interestingly, one tree (F5) from Fasa in southern Iran is in the clade with *J. polycarpus*. The other 6 trees from Fasa are in a group with *J. p. var. turcomanica* (Fig.4) with 2 trees from Kuhbanan (U0, U1). However, all of these trees seem to be a little different from typical *J. p. var. turcomanica* from the Kopet Mtns., Turkmenistan (Fig. 4). There is a large group of trees from southern Iran in the large clade with *J. seravschanica* (Kazakhstan and Pakistan). However, there are lots of differences within this clade. Three trees from Rabor [R3(R2, R4)] are well supported as a distinct clade (Fig. 4). All 5 trees from Khabr form a well-supported clade.

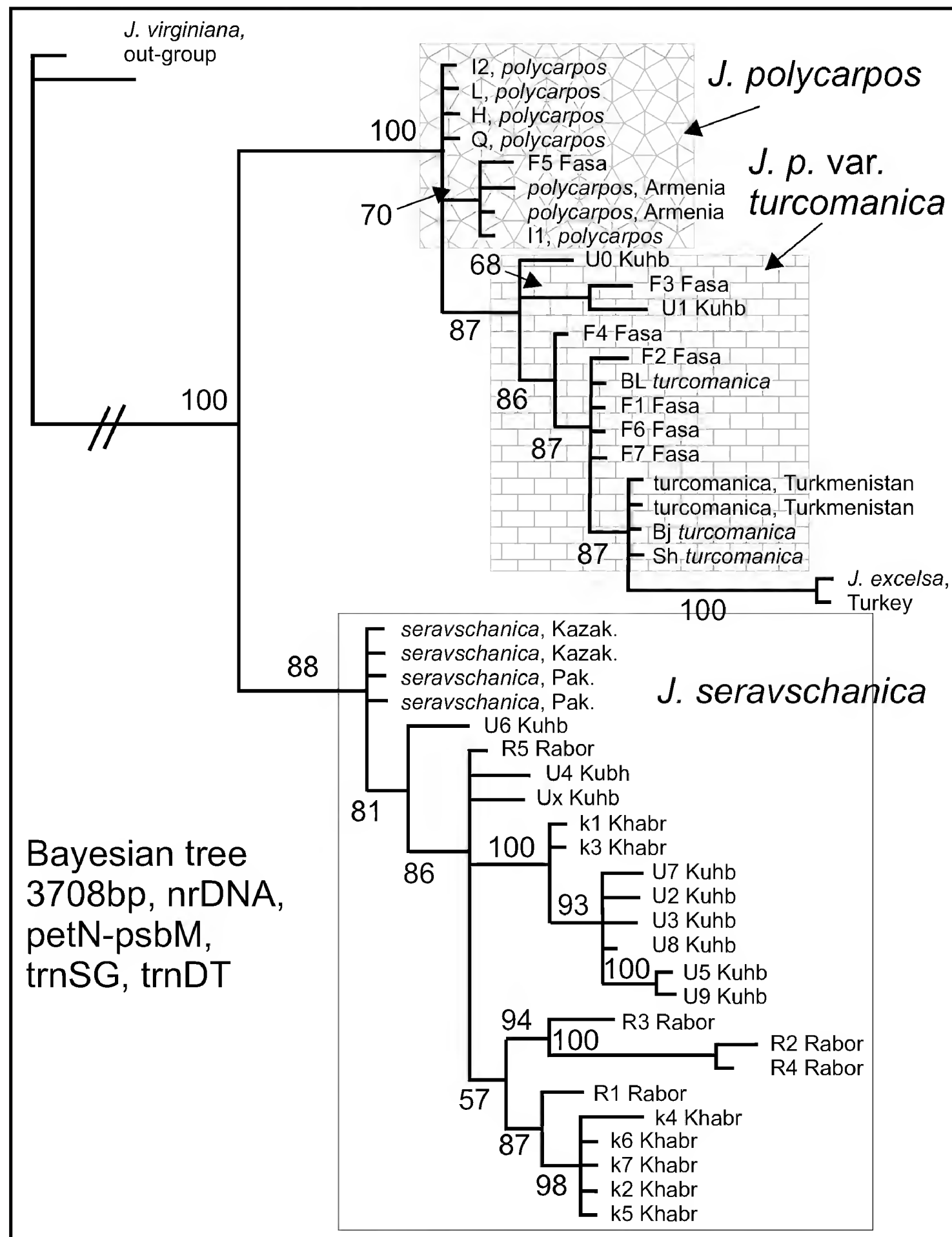


Figure 4. Bayesian tree of *Juniperus* from Iran plus exemplars of *J. excelsa*, *J. polycarpus*, *J. p. var. turcomanica*, and *J. seravschanica*. Numbers at branch points are posterior probabilities as percent.

To examine the magnitude of DNA differences among OTUs, a minimum spanning network was constructed using 124 MEs (mutational events = substitutions + indels). Five trees from Fasa appear to be the very uniform (Fig. 6) and differ by only 1 or 2 MEs from *J. p. var. turcomanica*. But F5 differs by 5 MEs from *J. p. var. turcomanica* and only 3 MEs from *J. polycarpus* (Fig. 5). F3 differs by 4 MEs. These trees may be relicts of ancient (or modern) hybridization between *J. polycarpus* and *J. p. var. turcomanica* in the Fasa area. Whether typical *J. polycarpus* occurs in the Fasa area has not been determined. Although tree F5 is in a clade with typical *J. polycarpus* from Armenia (Fig. 4), support is only 70%. The minimum spanning network indicates that F5 might be a hybrid.

Two of the trees from Kuhbanan (U0, U1) are grouped with the *J. turcomanica* from Fasa (Fig. 5), whereas 7 Kuhbanan trees are linked to *J. seravschanica* (Fig. 6). Two Kuhbanan trees (Ux, U4) are linked with Khabr samples (Fig. 5). The Kuhbanan population appears to be very diverse. This may be due to hybridization. The nrDNA (ITS) sequences indicated that most of the trees from Kuhbanan appear to be hybrids (see boxes in Fig. 5) or multi-copy due to incomplete lineage sorting. The Rabor trees are somewhat different and 2 trees (R4, R2) differ by 10 MEs from other Rabor trees (Fig. 5). This is also reflected in the Bayesian tree (Fig. 4) where R4 and R2 form a distinct clade.

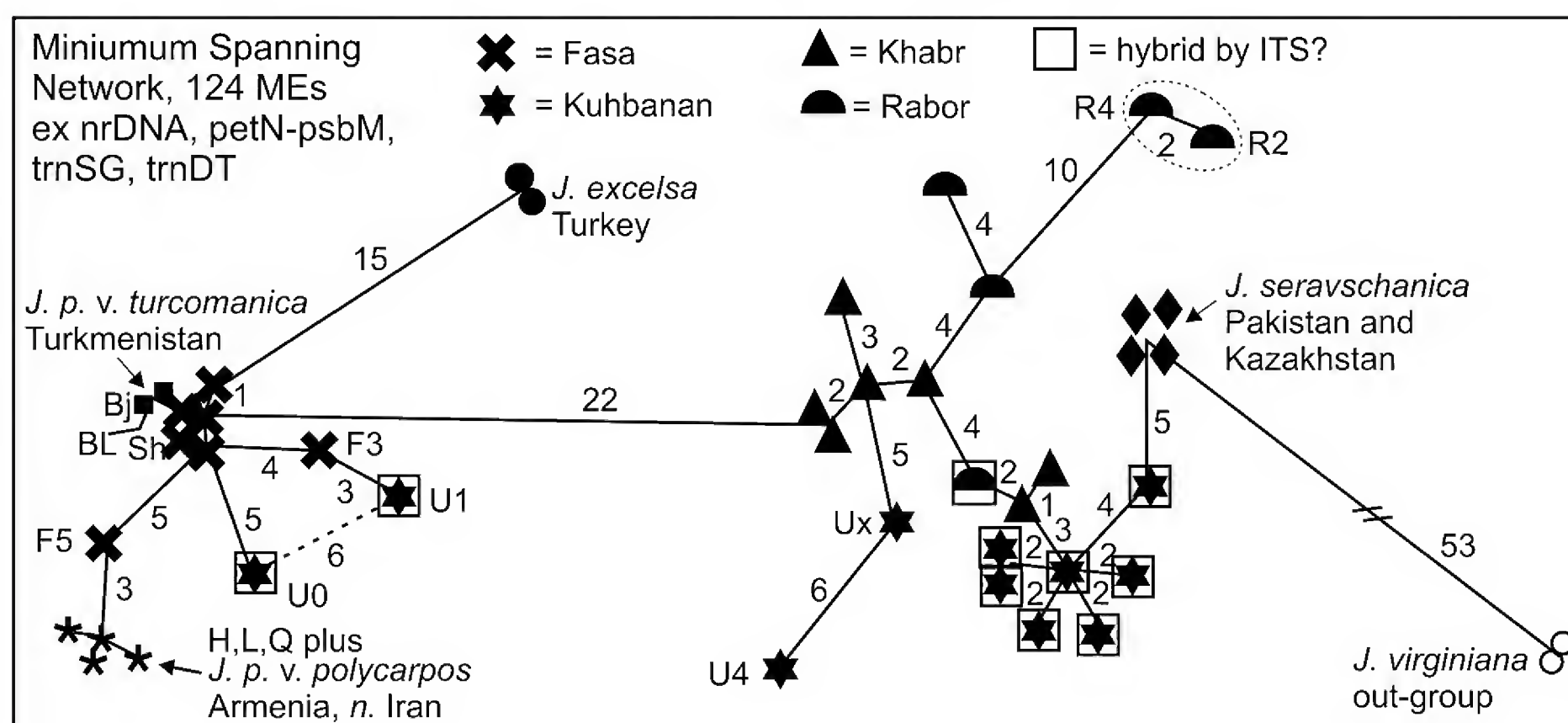


Figure 5. Minimum spanning network based on 124 MEs (mutational events = substitutions + indels). The numbers next to the lines are the number of MEs for that link.

As previously mentioned, the leaf oils of the new samples were found to have 2 chemotypes: high and low cedrol. In *Juniperus* (and most Cupressaceae), the leaf essential oils and the heartwood oils are usually very different in composition. The wood oil composition is conserved and composed of only sesquiterpenes: cf. cedrol, widdrol, α -cedrene, β -cedrene and cis-thujopsene (Adams, 1991, 2011). The leaf oils are very diverse in *Juniperus* with large amounts of monoterpenes: cf. α -pinene, sabinene, δ -3-carene, limonene, camphor, 4-terpineol, bornyl acetate; sesquiterpenes: caryophyllene, elemol, cadinenes, muurolenes, and diterpenes: cf. manool, manool oxide, abietadiene, abietatriene, trans-totarol, etc. In the western hemisphere, the wood and leaf terpene synthesis pathways appear to be almost exclusively expressed in different tissues. However, in the multi-seeded junipers of section *Sabina* in the eastern hemisphere (*J. excelsa*, *J. foetidissima*, *J. polycarpus*, *J. procera*, *J. seravschanica*, *J. thurifera*), it is common to find wood oil components in the leaf oil (but not *vice versa*). In fact, cedrol can be the major component in the leaf oils as reported by Adams and Hojjati (2013) for: *J. excelsa* - 25.4%; *J. polycarpus* - 30.3% and *J. seravschanica* - 22.7 - 13.8%. To examine the correlation of high cedrol with taxonomy, the individuals with high cedrol are circled in Fig. 6. The individuals with high cedrol are scattered

Nearly all samples from Kuhbanan were polymorphic in their nrDNA (ITS) chromatograms, implying either hybridization or incomplete lineage sorting with mixed nrDNA. Clearly the dynamics of *Juniperus* taxa in southern Iran are complex. Additional research utilizing other nuclear genes (ex. ABI3, 4CL, etc.) should aid in understanding this complex.

ACKNOWLEDGEMENTS

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A new variety of *Astragalus hyalinus* (Fabaceae) from Wyoming

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ABSTRACT

Astragalus hyalinus M. E. Jones var. *glabratus* Evert ex Dorn (Fabaceae) from the northwest edge of the range of the species in Park County, Wyoming is described. The new variety has the dorsal surface of the petals glabrous unlike the typical variety which has the dorsal surface of the petals conspicuously villous. The new variety tends to have fewer ovules also. Published on-line www.phytologia.org *Phytologia* 96(1): 26-27 (Jan. 8, 2014). ISSN 030319430

KEY WORDS: *Astragalus hyalinus*, glabrous petals, Wyoming

In 1981 Erwin Evert discovered a slightly disjunct population of *Astragalus hyalinus* M. E. Jones on the northwest edge of the species range with the dorsal surface of the petals glabrous unlike the villous dorsal petal surface in the rest of the range of the species. When Evert completed his annotated catalog and atlas of plants of the Yellowstone area (Evert 2010), he treated these plants as “++***Astragalus hyalinus* Jones var. *glabratus* undescribed variety.**” The double plus meant an endemic taxon confined to the Greater Yellowstone Area. He gave the range as Cedar and Sheep Mountains west of Cody, Park Co., Wyoming. Four months after completing his catalog and atlas, he was tragically killed by a grizzly bear about 40 kilometers west of the *Astragalus* populations. The purpose of this paper is to validate the name Evert provided for this variant of *Astragalus hyalinus*.

***Astragalus hyalinus* M. E. Jones var. *glabratus* Evert ex Dorn, var. nov.**

Type: USA, Wyoming, Park Co., Cedar Mountain ca. 5 mi SW of Cody, T52N R102W Section 8 NW ¼, rocky calcareous ridge, 7600 ft, 30 June 1989, R. Dorn 5023 (HOLOTYPE: RM, ISOTYPE: MO; PARATYPE: USA, Wyoming, Park Co., Cedar Mt., ca. 5 mi SW of Cody, 7 July 1981, E. F. Evert 3012 NY).

Differt a var. *hyalinus* petalis dorsaliter glabris et ovulis vulgo paucior (var. *glabratus* 6-8, var. *hyalinus* 8 vel 9). Differing from var. *hyalinus* in having the petals glabrous dorsally and the ovules commonly fewer (var. *glabratus* 6-8, var. *hyalinus* 8 or 9). Despite the number of ovules, the seeds are often only one or two. The petals of var. *glabratus* sometimes have a short tuft of hairs at the very base. In var. *hyalinus* the petals are conspicuously villous dorsally. *Astragalus hyalinus* differs from other trifoliate *Orophaca Astragali* by either the long calyx tube, 5.5 mm or more long, or the fiddle-shaped banner. It generally blooms later than the other *Orophaca Astragali*. The species ranges from southern Montana and southwest South Dakota south through Wyoming (except the southwest), western Nebraska, northwest Kansas, and northeast Colorado. Variety *glabratus* is known only from Park County, Wyoming 8-24 km southwest of Cody on Cedar and Sheep mountains from 1900 to 2310 m (6500-7600 ft) on limestone outcrops. The closest known populations of var. *hyalinus* are about 75 km to the east.

ACKNOWLEDGMENTS

I thank B. E. Nelson for searching for possible Evert collections of var. *glabratus* at RM.

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**The leaf essential oil of *Juniperus formosana* (Taiwan) compared
with *J. mairei* (Gansu, China) and *J. jackii***

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ABSTRACT

The compositions of the essential oils of *Juniperus formosana*, *J. jackii* and *J. mairei* are presented. The volatile leaf oil of *J. formosana* (Taiwan) is dominated by α -pinene (44.0%), with moderate amounts of β -pinene (4.2%), myrcene (6.4%), β -phellandrene (5.3%), δ -cadinene (2.9%), germacrene D-4-ol (3.8%) and α -cadinol (4.1%). The oil shares the large concentration of α -pinene with *J. mairei* but has fewer compounds. *Juniperus formosana* contains several unique compounds, including sibirene, that is rare in *Juniperus* leaf oils. Each of these 3 species have a unique oil composition that broadly supports their phylogenetic differences. Published on-line www.phytologia.org *Phytologia* 96(1): 28-32 (Jan. 8, 2014). ISSN 030319430

KEY WORDS: *Juniperus formosana*, *J. f. var. mairei*, *J. mairei*, *J. jackii*, leaf essential oils, terpenes, taxonomy.

Recently, Adams and Schwarzbach (2012) have shown that *Juniperus formosana* Hayata and *J. formosana* var. *mairei* (Lemee and H. Lev) R. P. Adams and C.-F. Hsieh are not as closely related as previously thought. In fact, *J. f. var. mairei* (Gansu, China) was found in a clade (Fig. 1) with *J. jackii* (Rehder) R. P. Adams (North America), not with *J. formosana* (Taiwan). A minimum spanning network revealed (Adams and Schwarzbach, 2012) that *J. f. var. mairei* differs by 13 MEs from *J. formosana* (Taiwan) and 20 MEs from *J. jackii* (Fig. 2). Although Adams (2011) recognized *J. f. var. mairei*; recently, Adams and Schwarzbach (2012) recognized *J. mairei* as a distinct species.

The composition leaf essential oil of *J. mairei* (Gansu) have been reported (as *J. formosana* in Adams, Zhang and Chu, 1995, Adams, 2000) and the leaf essential oil of *J. jackii* was published by Adams, 2013 and Adams et al., 2010. Yu et al. (1994) reported on the leaf oil of *J. formosana* (*J. mairei*) from the China mainland. However, there appears to be no report on the leaf essential oil of *J. formosana* var. *formosana* from Taiwan. *Juniperus formosana* is endemic to Taiwan, except for one report of its occurrence in Kushan across the Taiwan (Formosa) Strait from Taiwan, on the China mainland (Adams, 2011). Adams, Zhang and Chu (1995) reviewed the literature, most of which reports on wood oil compositions.

The purposes of this paper are to report on the composition of the leaf essential oil of *J. formosana* from Taiwan and to compare it with the oils of two related species, *J. jackii* and *J. mairei*.

[illegible]

RESULTS AND DISCUSSION

The volatile leaf oil of *J. formosana* (Taiwan) is dominated (Table 1) by α -pinene (44.0%), with moderate amounts of β -pinene (4.2%), myrcene (6.4%), limonene (3.5%), β -phellandrene (5.3%), δ -cadinene (2.9%), germacrene D-4-ol (3.8%) and α -cadinol (4.1%). The oil shares the large concentration of α -pinene with *J. mairei* but has fewer compounds (Table 1). *Juniperus formosana* contains several unique compounds: naphthalene, β -cubebene, sibirene, trans-cadina-1(6),4-diene, sandaracopimarinal and trans-totarol. Sibirene is rare in *Juniperus* leaf oils (Adams, 2011).

The oil of *J. mairei* is more similar to *J. formosana* than to *J. jackii* (Table 2), reflecting the minimum spanning network (Fig. 2), rather than the Bayesian tree (Fig. 1). However, it contains several unique (to this small set of 3 species) compounds: 3-me-3-butenol butyrate, unknown terpene alcohol (at KI 1092), borneol, unknown 1198, prenyl hexanoate (1.1%), (E)-methyl iso-eugenol, (E)-nerolidol, geranyl butanoate and oplopenone (Table 1).

In contrast, the oil of *J. jackii* has a moderate amounts of α -pinene (16.1%), δ -3-carene (17.9%), β -phellandrene (13.4%), myrcene (3.2%), limonene (6.6%), terpinolene (3.2%), and germacrene D (4.1%). Its oil contains several unique compounds: δ -3-carene, p-mentha-1,5-dien-8-ol, thymol, methyl ether, methyl myrtenate, α -terpinyl formate, β -elemene, γ -muurolene, α -cadinene, germacrene B, salvial-4(14)-en-1-one, cyclohexadecanolide, manoyl oxide, abietatriene and isoabienol.

These 3 species have unique oil compositions that broadly support their phylogenetic differences seen in DNA sequencing (Figs. 1, 2).

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Table 1. Comparison of the leaf oils of *J. formosana* (Taiwan), *J. mairei* (Gansu, China) and *J. jackii* (North America). Compounds in bold face appear to separate taxa.

KI	Compound	<i>formosana</i> Taiwan	<i>J. mairei</i> Gansu	<i>jackii</i> N. Am.
851	(E)-2-hexenal	1.2	t	0.2
921	tricyclene	t	t	t
924	α -thujene	t	0.1	t
932	α-pinene	44.0	55.5	16.1
945	α -fenchene	t	t	0.3
946	camphene	0.4	0.5	0.3
953	thuja-2,4-diene	-	t	-
961	verbenene	-	1.4	0.3
969	sabinene	0.1	0.2	0.1
974	β -pinene	4.2	2.5	1.9
988	myrcene	6.4	5.7	3.2
1001	δ -2-carene	2.6	0.7	0.2
1002	α -phellandrene	0.7	0.5	2.2
1008	δ-3-carene	-	-	17.9
1020	p-cymene	0.3	1.1	1.1
1024	limonene	3.5	2.4	6.6
1025	β-phellandrene	5.3	2.3	13.4
1044	(E)- β -ocimene	0.2	-	0.3
1054	γ -terpinene	t	-	0.1
1061	3-me-3-butenol butyrate	-	0.4	-
1086	terpinolene	0.6	0.7	3.2
1092	C10-OH ,96,109,137,152	-	1.3	-
1114	endo-fenchol	-	0.7	-
1118	cis-p-menth-2-en-1-ol	0.1	-	0.2
1122	α -campholenal	-	0.3	0.2
1132	cis-limonene oxide	-	-	0.1
1136	trans-pinocarveol	-	0.3	0.2
1140	trans-verbenol	0.1	0.5	0.2
1145	camphene hydrate	-	0.3	-
1165	borneol	-	0.3	-
1166	p-mentha-1,5-dien-8-ol	-	-	0.4
1174	terpinen-4-ol	t	0.4	0.7
1178	naphthalene	0.6	-	-
1179	p-cymen-8-ol	-	0.2	0.3
1186	α -terpineol	0.1	0.6	0.3
1195	myrtenol	-	0.3	0.4
1198	C10-al,95,121,139,154	-	0.5	-
1204	verbenone	-	0.1	0.3
1215	trans-carveol	-	0.1	0.4
1223	citronellol	0.3	0.4	-
1232	thymol, methyl ether	-	-	0.2
1235	cis-chrysanthenyl acetate	-	0.2	-
1249	piperitone	-	0.8	-
1260	3-me-3-butenol hexanoate	0.1	0.9	-
1284	bornyl acetate	0.7	1.1	0.5
1292	prenyl hexanoate	-	1.1	-
1293	methyl myrtenate	-	-	0.2
1302	α -terpinyl formate	-	-	1.0
1324	myrtenyl acetate	-	t	1.6
1332	cis-piperitol acetate	-	0.2	-
1346	α -terpinyl acetate	-	0.1	0.9
1374	α -copaene	0.3	0.2	-
1385	trans-myrtanyl acetate	-	-	t

KI	Compound	<i>formosana</i> Taiwan	<i>mairei</i> Gansu	<i>jackii</i> ¹ N. Am.
1379	geranyl acetate	-	t	-
1387	β -cubebene	0.2	-	-
1391	β -elemene	-	-	0.3
1400	sibirene	0.7	-	-
1417	(E)-caryophyllene	0.9	0.7	0.4
1452	α -humulene	0.4	0.4	0.5
1465	cis-muurolo-4(14),5-diene	-	-	t
1473	isobornyl-n-butanoate	-	0.2	-
1475	trans-cadina-1(6),4-diene	0.2	-	-
1478	γ -muurolene	0.3	-	t
1480	germacrene D	1.5	1.2	4.1
1491	(E)-methyl iso-eugenol	-	0.2	-
1493	epi-cubebol	0.6	-	0.3
1499	γ -muurolene	-	-	0.6
1500	α -muurolene	0.6	0.1	-
1503	germacrene A	-	-	t
1513	γ -cadinene	1.3	2.2	1.2
1514	cubebol	1.3	-	-
1522	δ -cadinene	2.9	0.5	2.2
1537	α -cadinene	-	-	0.2
1548	elemol	-	-	t
1559	germacrene B	-	-	0.5
1561	(E)-nerolidol	-	0.2	-
1561	geranyl butanoate	-	0.4	-
1574	germacrene D-4-ol	3.3	0.6	0.9
1582	caryophyllene oxide	0.3	0.5	0.2
1592	salvial-4(14)-en-1-one	-	-	0.1
1608	humulene epoxide II	0.3	0.4	t
1627	1-epi-cubenol	0.8	-	1.5
1638	epi- α -cadinol	1.5	0.6	0.7
1640	epi- α -muurolol	1.5	0.6	0.8
1644	α -muurolol	0.6	t	0.4
1652	α -cadinol	4.1	0.6	2.0
1685	germacra-4(15),5,10(14)-trien-1-al	0.6	-	0.3
1688	shyobunol	-	-	t
1715	(2Z,6E)-farnesal	0.4	1.0	-
1739	oplopenone	-	0.3	-
1933	cyclohexadecanolide	-	-	0.1
1987	manoyl oxide	-	-	0.2
2022	abieta-8,12-diene	-	-	-
2055	abietatriene	-	-	0.3
2056	manool	1.1	-	0.6
2087	abietadiene	-	-	-
2105	isoabienol	-	-	0.2
2184	sandaracopimarinal	0.2	-	-
2314	trans-totarol	0.2	-	-
2331	trans-ferruginol	0.5	-	t

KI = Kovat's Index on DB-5(= SE54) column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Two new cryptic species of *Menodora* (Oleaceae) from southern Coahuila, Mexico

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ABSTRACT

Two novel cryptic taxa of *Menodora* are described from southern Coahuila, Mexico: ***M. chumleyi*** B.L. Turner, **sp. nov.**, and ***M. geohintonii*** B.L. Turner, **sp. nov.**; the former relates to *M. coulteri*, the latter to *M. helianthemoides*. Both of the novel taxa belong to a DNA clade having pubescent stylar shafts, the only plants in the genus known to possess such pubescence, except for an anomalous plant of *M. muelleriae*. Maps showing the distributions of the several taxa are provided, along with photographs of *M. geohintonii* growing in the field. Published on-line www.phytologia.org *Phytologia* 96(1): 33- 40 (Jan. 8, 2014). ISSN 030319430

KEY WORDS: Oleaceae, *Menodora*, Mexico, Coahuila, *M. coulteri*, *M. helianthemoides*.

MENODORA CHUMLEYI B.L. Turner, **sp. nov.** **Fig. 1**

Resembling ***M. coulteri*** but a taller, more intricately branched plant having much larger corollas and pubescent stylar shafts.

Suffruticose herbs or intricately branched **shrublets** to 30 cm high. **Stems**, lowermost, markedly woody, up to 1.5 mm across; mid-stems 1.5-4.0 mm across, pubescent with minute, downwardly directed hairs 0.1-0.2 mm long. **Leaves** linear-lanceolate, 10-25 mm long, 2-3 mm wide, minutely ciliate along the margins, if at all, their apices acute. **Flowers** reflexed with age. **Calyces** 7-10 mm long, having ca 12 ciliate, linear, lobes 6-8 mm long. **Corollas** yellow, glabrous, except for the throat of the tube; tubes ca 3 mm long; lobes 10-14 mm long, 3-5 mm wide. **Stamens** exerted for 10-14 mm, the filaments sparsely pubescent; anthers ca 2 mm long. **Styles** exerted for 10-12 mm, the shafts decidedly pubescent. **Fruiting pedicels** 8-10 mm long, recurved. **Capsules** circumscissile, 4-5 mm high, and as wide, glabrous; seeds 1/locule, obovate, ca 4 mm long, 2 mm wide, the surfaces smooth and shiny.

TYPE: MEXICO. COAHUILA: Mpio. Arteaga, Sierra Zapaliname, 2505 m, “Bushy limestone hillside.” 22 Jun 1992, *G.B. Hinton et al.* 22066 (Holotype : TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. COAHUILA: Mpio. Arteaga, Sierra Zapaliname, 2430 m, 19 May 1990, *Hinton et al.* 20240 (TEX). **Mpio. Saltillo**, ca 6 km W of Saltillo, “E. extremity of the Sierra de la Vega, at and below Estacion Microondas La Vega,” 25 25 N, 101 05 W, 1800-2000 m, 30 Mar 1973, *Johnston, Wendt & Chiang* 10501 (LL); ca 11 mi W of Saltillo, “near Est. Vega microwave tower,” 27 Sep 1974, *Rollins & Rollins* 7467 (TEX).

Early on, I identified all of the above sheets as ***M. coulteri***, which they indeed resemble. Closer inspection and the DNA studies by Chumley, along with the detection of hairs on stylar shafts, have led to the cryptic species proposed here. Interestingly, *Johnston et al.* 10501 (LL) was designated as the holotype of Chumley’s intended *M. henricksonii*, this not annotated as such at TEX, hence my ignorance of Chumley’s eponym and my selection of a different holotype.

The distribution of ***M. chumleyi*** and its closest morphological cohort, ***M. coulteri***, is shown in **Map 1**.

MENODORA GEOHINTONII B.L. Turner, **sp. nov.** Fig. 2

Resembling **M. helianthemoides** but the stem pubescence more nearly pilose (vs minutely hispid), the leaves smaller, flowers larger, and stylar shafts pubescent.

Suffruticose herbs or dwarf subshrubs 5-20 cm high. **Stems** (lower) decidedly woody, 3-4 mm across, at mid-stem decidedly pubescent with spreading hairs 0.3-0.5 mm long. **Leaves opposite**, 8-12 mm long, 2.5-4.0 mm wide; petioles 1-2 mm long; blades linear-elliptic to linear-oblongate, uninervate, grading into the blades, pubescent along the margins like the stems. **Flowers** reflexed with age. **Calyces** 6-7 mm long having 12-14, markedly ciliate, linear lobes 7-8 mm long. **Corollas** yellow, glabrous except for the corolla throat; tubes ca 3 mm long; lobes 10-12 mm long, 5-6 mm wide. **Stamens** exerted for 5-7 mm, the filaments sparsely pubescent; anthers yellow, ca 2 mm long. **Styles** exerted for 8-10 mm, the shafts decidedly pubescent with short stiff hairs, the stigmatic surfaces orbicular, ca 0.5 mm across. **Fruiting pedicels** 4-6 mm long, recurved. **Capsules** 4-5 mm high, and as wide, glabrous; seeds (immature), seemingly tuberculate.

TYPE: MEXICO. COAHUILA: Mpio. Ramos Arizpe, Sierra San Jose de los Nuncios, "Limestone rockslide." 1375 m, *G.B. Hinton et al. 21048* (Holotype: TEX).

In my overview of North American **Menodora** (Turner 1995), I placed the above type specimen into my concept, at the time, of *M. magniflora* (Steud.) B.L. Turner, this treated as a variety of **H. helianthemoides** by Steyermark (1932), who distinguished it from the latter by its inconspicuous, "short or closely appressed hairs," and larger corollas (14-17 mm long vs 9-14 mm). I did, however, call attention to its resemblance to **M. tehuacana** B.L. Turner, but failed, at the time, to note its pubescent stylar shafts. Chumley (2007) placed the type of *H. magniflora* in synonymy with **M. helianthemoides** proper, which I now follow; he subsequently examined DNA of the *Hinton 21048* collection, annotating this with the following cryptic notation "Menodora helianthemoides Bonpl. in morphology...Galeana cryptoclade molecularly..." The "Galeana clade" presumably consists of **M. gypsophila** and **M. muelleriae**.

At the time of Chumley's research, I called his attention to the anomalous, geographically remote, type specimen and suggested I might anoint the plant as **M. chumleyi**, if additional collections came to light. Regardless, my reexamination of the type concerned revealed several novel features that distinguished the plant from **M. helianthemoides**, including corolla size, stem pubescence and that of the stylar shaft, which possessed stiff short hairs, as does **M. chumleyi**, described above, the latter consisting of 4 specimens all annotated by Chumley as **M. coulteri** morphologically but molecularly belonging to the Galeana cryptic clade. I take both **M. chumleyi** and **M. geohintonii** to be cryptic species belonging to the Galeana clade, as noted by Chumley. Interestingly, all of the plants concerned possess pubescent stylar shafts, to my knowledge the only such plants within **Menodora** to have this feature (with the exception of a single sheet of **M. muelleriae** [*Hinton et al. 25349*] that possessed only 1 or 2 hairs on its stylar shafts).

I have not examined the type of *Menodora henricksonii* var. *confusa*, but I assume that Chumley's type is correctly positioned. However, typical plants of **C. coulteri** occur in the vicinity of Parras, Coahuila (e.g. *Cowan 3623*, TEX), the latter plant w/o corollas, but possessing the stem pubescence of **M. coulteri**, this not similar to that of the larger flowered plant having longer spreading hairs (*Hinton 21048*) that characterize my **M. geohintonii**. In short, it is possible that the type of *M. h.* var. *confusa* represents yet another cryptic species having smaller flowers, shorter stem hairs as well as pubescent stylar shafts, these confined to the vicinity of Parras, the latter an area known for its local endemics.

Finally, I should note that I informed George Hinton, who recently inquired about the identification of *Hinton 21048*, hence my restudy of the complex, that I would provide his eponym to the cryptic species IF he would revisit the site and report upon the population concerned, and perhaps obtain

suitable photographs. This he has done, noting that on revisiting Sierra San Jose de los Nuncios in December of 2013, 29 plants were found on the east slope of the Sierras, and 3 on the west slope. “Many were in the shade of Pine and Dasyllirion. The largest plant (and the one with the only flower, thanks to the late November rains) was about 30 cm in diameter and about 25 cm high, pictured below with Agave lechuguilla.” He also notes in his communication that the plants height as given on the type label is in error. It should have read 0.05 m., not “0.5 m.”

Distribution of **M. geohintonii** and its closest morphological cohort, **M. helianthemoides** (in my opinion), is shown in **Map 2**.

ACKNOWLEDGEMENTS

My thanks to Nancy Elder, librarian, Univ. of Texas, for bibliographic assistance and to Tim Chumley for discussions relating to his doctoral study of the complex during his studies at Texas. Tom Wendt kindly scanned type material. George Hinton, at my request, kindly re-collected specimens of **M. geohintonii**, along with field photographs; and Jana Kos, my long-time editorial colleague, provided helpful input.

The above paper was written and ready for press, following a diligent search for Chumley’s Doctoral Thesis, this reportedly deposited in 2007 in the Univ. of Texas Library, but not to be found; his excellent, detailed, study was subsequently provided to me by my colleague Prof. Beryl Simpson, this from sources other than the library. I was pleasantly surprised to find that Chumley too recognized both of the taxa proposed herein, although not recognizing these as “cryptic” and providing them with different names: *M. henricksonii* var. *henricksonii* (for my **M. chumleyi**) and *M. h.* var. *confusa* (for my **M. geohintonii**, at least in part). I was unaware that these names had been proposed, largely because Chumley had not annotated the sheets concerned as such in the TEX herbarium. Regardless, the present contribution was undertaken without access to his study, and I prefer my formal names over those proposed by him.

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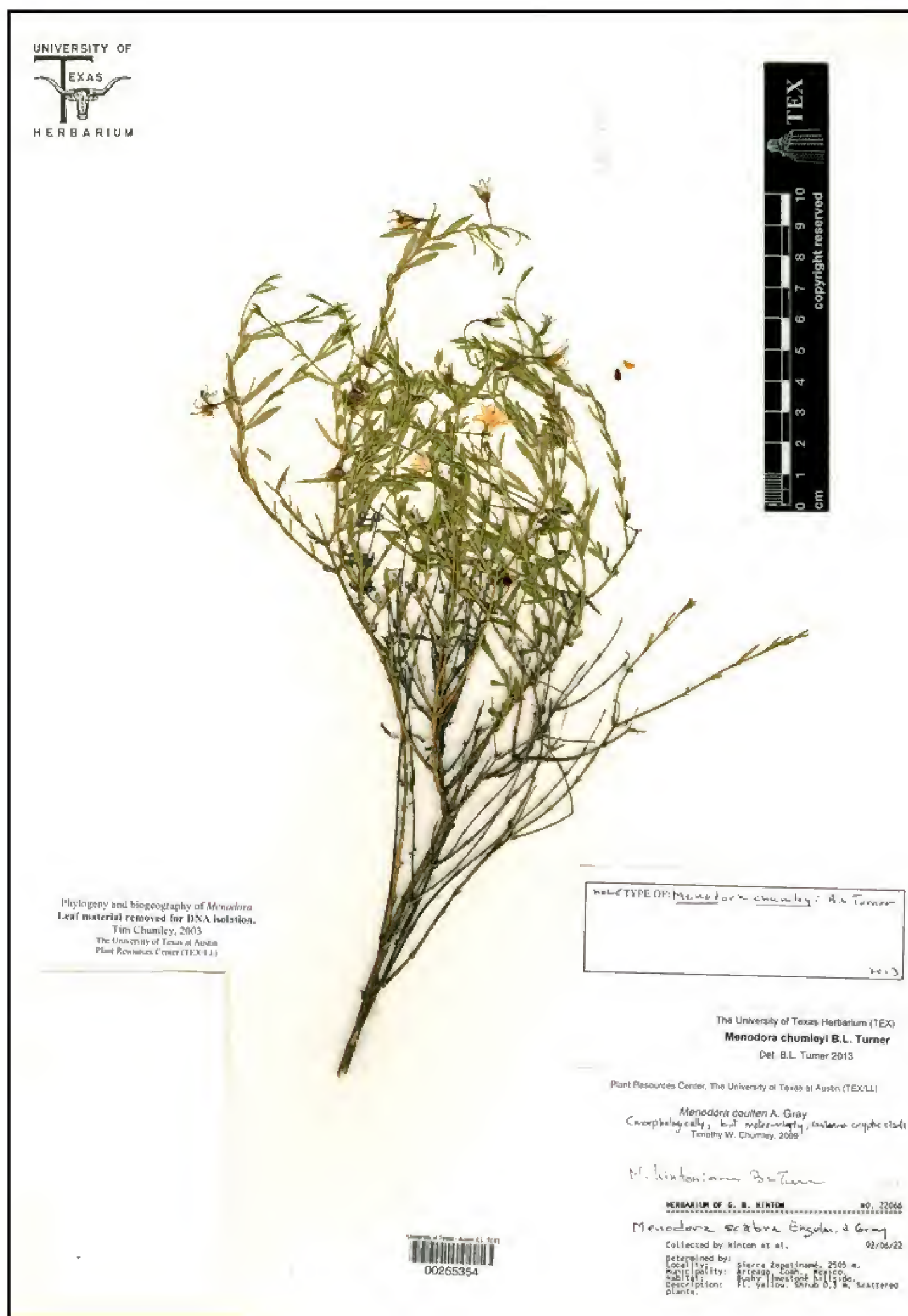


Fig. 1. *Menodora chumleyi* (Holotype: TEX).

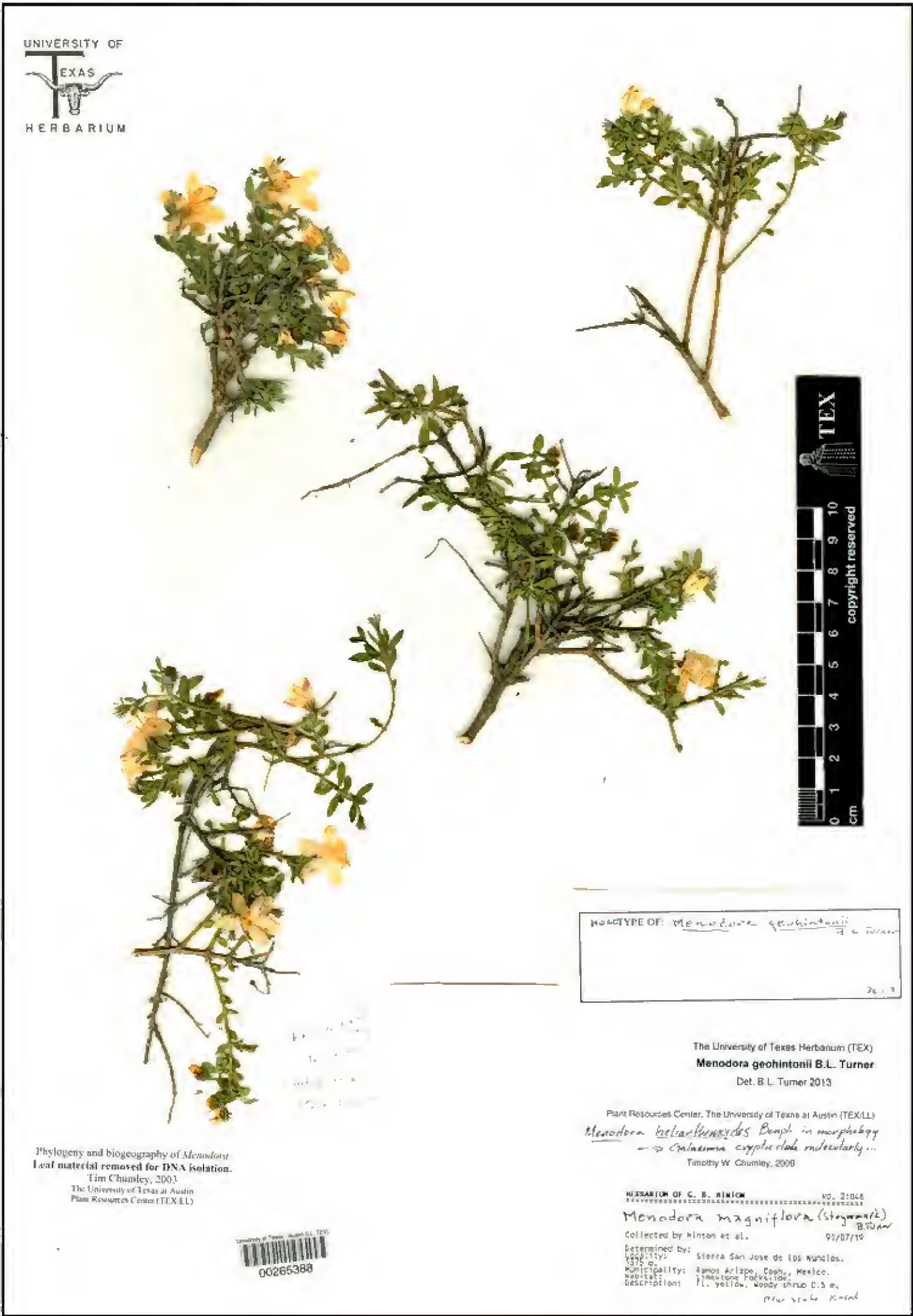


Fig. 2. *Menodora geohintonii* (Holotype: TEX).

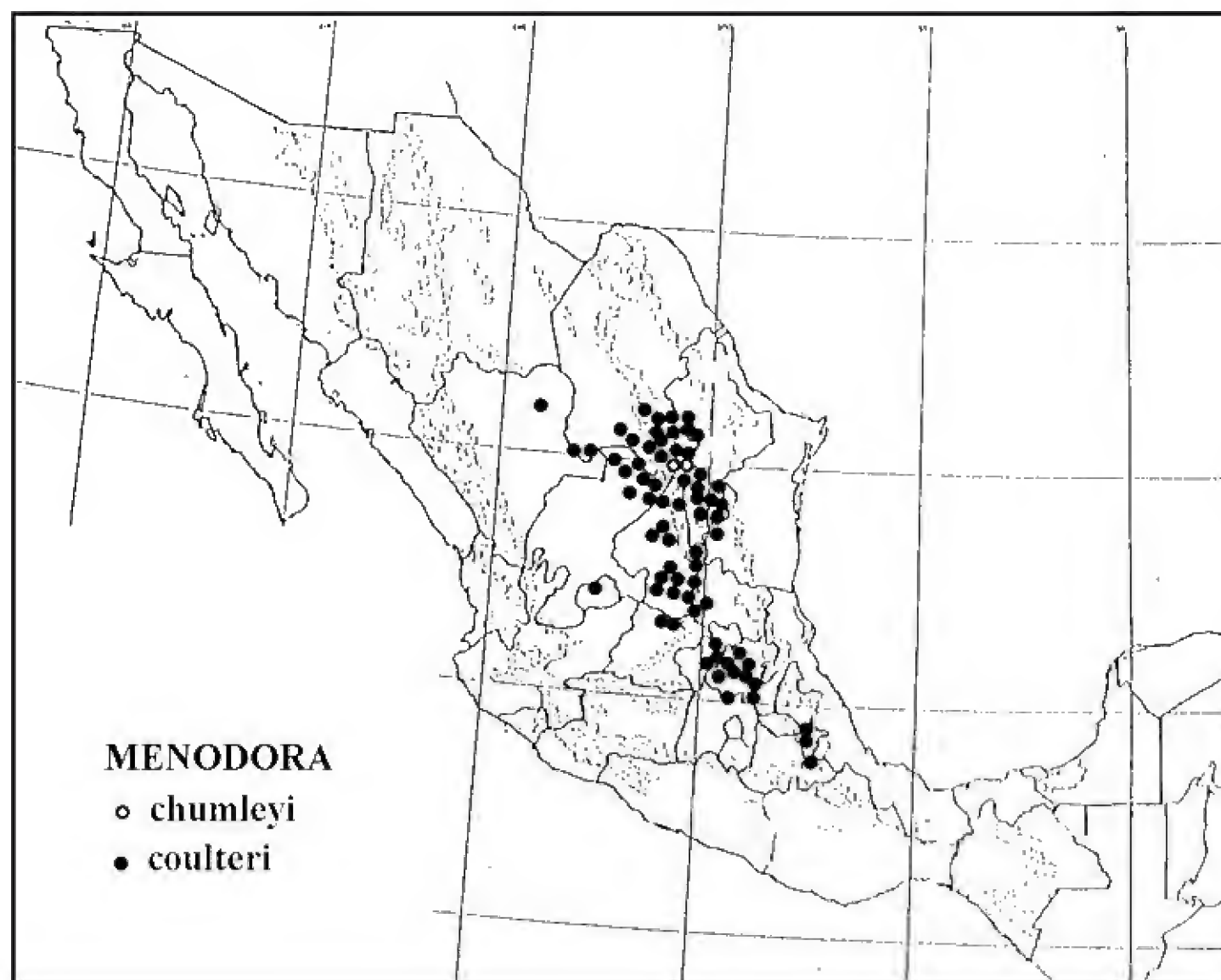


Fig. 3. Distribution of *M. chumleyi* and *M. coulteri*.

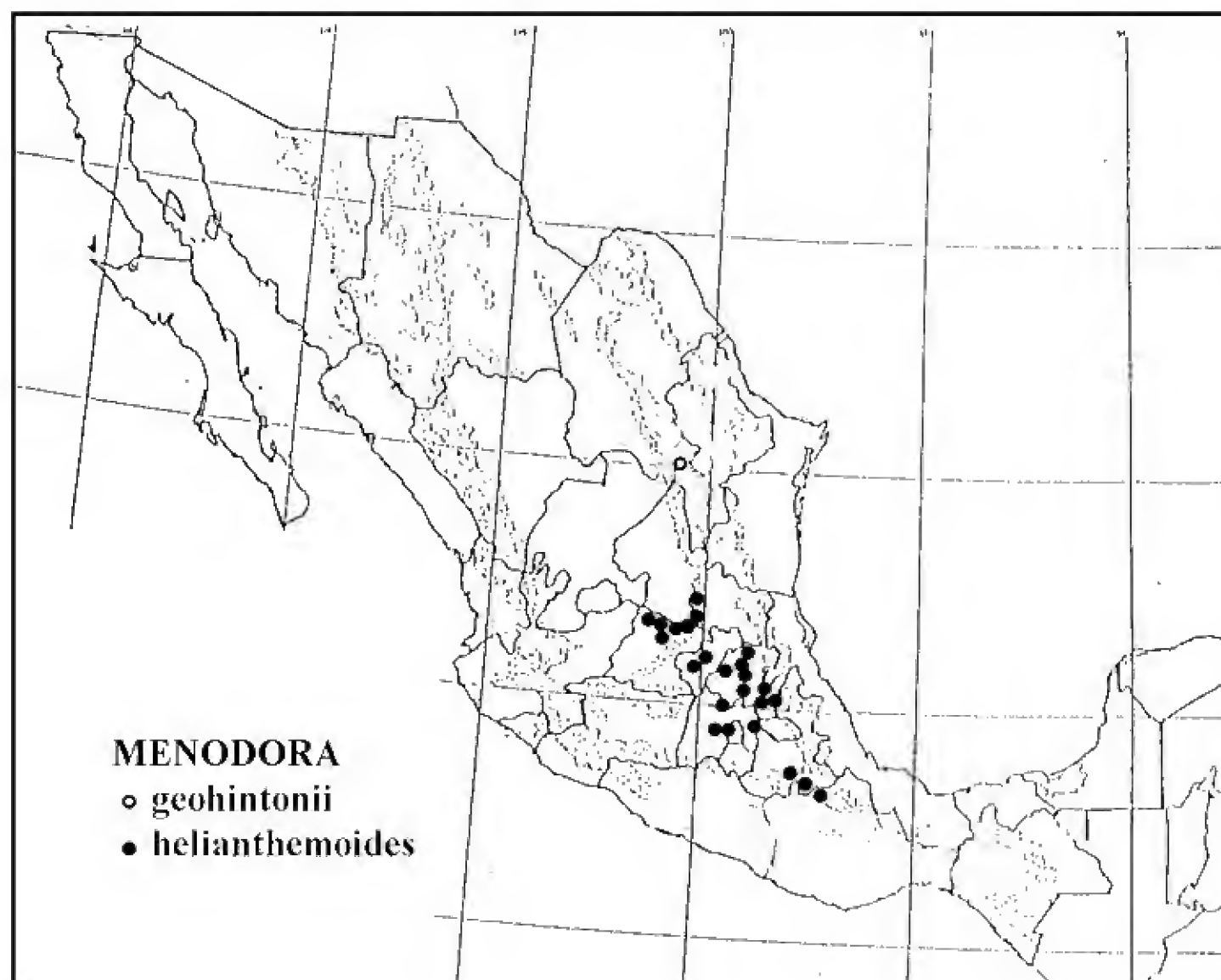


Fig. 4. Distribution of *M. geohintonii* and *M. helianthemoides*.



Fig. 5. *M. geohintonii* at type locality (growing behind *Agave lechugilla*).



Fig. 6. *M. geohintonii* (close-up of flower in Fig. 5).



Fig. 7. Hand-held plant of *M. geohintonii*.



Fig. 8. Type locality, most plants collected near the prominent Yucca in central foreground.



Fig. 9. West side of canyon where a few isolated plants were found.

**Questions regarding genus *Myzocyttium* (Oomycota, Straminipila) and its species:
Variation and identity of specimens in west-central Alabama**

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ABSTRACT

Myzocyttium is a poorly known genus of aquatic Oomycetes, formerly containing taxa parasitizing algae (mainly freshwater forms) or kinds of aquatic invertebrates. Recently, the genus has been restricted to species inhabiting algal hosts. *Myzocyttium proliferum*, *M. megastomum*, *M. netrii*, and *M. rabenhorstii* (a form of debatable placement, resembling *Lagenidium*) occur in Zygnemataceae, Desmidiaceae, or Cladophoraceae. *Myzocyttium* and *Lagenidium*, both traditionally placed in the Lagenidiaceae, can be morphologically and ecologically similar, and questions persist as to their morphological distinction and initial nomenclatural recognition; several later described genera must also be considered. Populations of *Myzocyttium* (found in *Spirogyra*) in western Alabama exhibited features, such as the form of the sporangial discharge tube, of either *M. proliferum* or *M. megastomum*, or were intermediate between them; these observations (and a common host occupied) challenge distinction of these species, and invoke questions of the role of genetics (vs. conditions encountered in host cells) in aspects of morphology. Objectives of our investigation included confirmation of specimens collected as belonging to genus *Myzocyttium*, as well as determination of species identity (or intermediacy). A key to species is presented. Published on-line www.phytologia.org *Phytologia* 96(2): 41-46 (April 1, 2014). ISSN 030319430

KEY WORDS: Conjugatae, Lagenidiales, *Myzocytiopsis*, *Pythium*, sporogenesis, *Syzygangia*, thallus.

The holocarpic Oomycete genus *Myzocyttium* Schenk (1858) has been historically placed in the Lagenidiales (e.g., Sparrow, 1960), more recently in the Pythiales (cf. Dick, 2001). *Myzocyttium* has been confused since its inception with *Lagenidium*—both genera based initially on similar (identical?) species, considered then to belong to genus *Pythium*. The date of publication of *Lagenidium* (usually credited as Schenk, 1859; e.g., Sparrow, 1960) may have been published in 1858 (or 1857) depending on when separates of Schenk's "1859" publication were issued (S. Redhead, personal communication). It remains uncertain which generic name (*Myzocyttium* or *Lagenidium*) appeared first—of concern if these genera are synonymous. Further nomenclatural complication is that neither name was formally combined with a specific epithet in initial publication. Morphological intermediacy has been observed between *Lagenidium* and *Myzocyttium* (Barron, 1976); distinction of these genera and priority of their names must be further sorted (Dick, 2001; Redhead, personal communication)—involving many species, and several segregate genera (e.g., *Syzygangia*)—not goals of our investigation, other than discussion. Our study deals with organisms (algal parasites) placed in *Myzocyttium*, as currently understood (cf. Dick, 2001). *Myzocyttium* may usually be distinguished from *Lagenidium* (as traditionally recognized) by a more regular, catenulate thallus (individual cells often developing a sub-spherical shape, and sometimes separated by distinct partitions) and by less differentiated gametangia (often without obvious fertilization tubes). The taxonomy of *Lagenidium*—considered (Karling, 1981) the largest genus of Lagenidiales—was drastically altered (taxa greatly reduced) by Dick (2001), and remains controversial (Blackwell et al., 2013).

TAXONOMIC SUMMARY AND BIOLOGICAL OCCURRENCE

The genus *Myzocyttium*—originally monotypic, based on an organism called "*Pythium proliferum*" by Schenk (1858)—underwent taxonomic expansion, followed by reduction of taxa. *Myzocyttium* was considered by Sparrow (1960) to have five species, and by Karling (1981) as many as 16. The genus was traditionally viewed as containing parasites of both green algae and animals (nematodes and rotifers).

More recently—in connection with recognition of several new genera (Dick, 1997; Dick, 2001)—*Myzocyttium* was restricted to algal parasites. The four species of *Myzocyttium* still recognized (Dick, 2001), all algal-inhabiting, are: *M. proliferum* Schenk (the type), *M. megastomum* De Wildeman, *M. rabenhorstii* (Zopf) Dick, and *M. netrii* (Miller) Dick. As for other possible taxa: *Myzocyttium irregulare* Petersen was thought to be synonymous with *M. megastomum* (Dick, 2001; Canter, 1947), or possibly to belong to *Lagenidium* (Fitzpatrick, 1930); *Myzocyttium lineare* Cornu is too poorly known for generic placement; and *M. proliferum* forma *marinum* Kobayashi & Ookubo (an apparent instance of marine occurrence) is doubtfully distinct from typical *M. proliferum* (cf. Johnson and Sparrow, 1961). *Myzocyttium globosum* Schenk and *M. anomalum* Dasgupta & John (possibly an illegitimate name) are considered synonyms of *M. proliferum* Schenk; see Dick (2001) and *Index of Fungi* (current) for lists of synonyms and excluded/doubtful names. No new species were found under *Myzocyttium* since Dick's (2001) coverage.

Myzocyttium proliferum, *M. megastomum*, *M. netrii*, and *M. rabenhorstii* parasitize freshwater algae, mostly “Conjugatae” (*Spirogyra*, *Mougeotia*, *Zygnema*, and certain desmids). The first two of these (*Myzocyttium*) species may also be found in Cladophoraceae (*Cladophora* or *Rhizoclonium*). *Myzocyttium rabenhorstii* (occurring in *Spirogyra*) and *M. netrii* (found in the “saccoderm desmid,” *Netrium*) were both originally described as species of *Lagenidium*. Placement of *Lagenidium netrii* Miller (1965) in *Myzocyttium* (Dick, 2001) seems morphologically appropriate. However, *Myzocyttium rabenhorstii* (Zopf) Dick (2001)—based on *Lagenidium rabenhorstii* Zopf (1878)—exhibits traits of traditional *Lagenidium* (e.g., an obvious fertilization tube) or of the segregate genus, *Syzygangia* (Dick, 1997). The generic border between *Lagenidium* and *Myzocyttium* being indistinct, their further evaluation—and reappraisal of related genera (*Syzygangia*, *Chlamydomyzium*, *Myzocytiopsis* and *Aphanomyopsis*)—is obviously advisable. Molecular-genetic study of these genera has been limited (Beakes and Sekimoto, 2009). *Myzocyttium*, if restricted to algal parasites (Dick, 2001), is yet to be analyzed in such investigations (difficult in obligate endo-parasites); as evident below, a range of thallus types is represented.

Sparrow's (1960) key to *Myzocyttium* taxa included parasites of plants or animals; Dick's (2001) treatment recognized only algal parasites, but offered no key—hence, our key to species below.

Key to species of *Myzocyttium* presently recognized

1. Thallus typically remaining one-celled.....*M. netrii*
1. Typically becoming multi-celled, the thallus often with a chain-like appearance.
 2. Thallus often more or less linear (sometimes tiered or irregular in part), catenulate; partitions between cells sometimes becoming plate-like; cells prone to attain a spheroidal shape.
 3. Sporangial discharge tube bulbous prior to exit from the cell, usually not especially elongate.....*M. megastomum*
 3. Discharge tube more uniformly cylindrical, sometimes elongate and extending well beyond the host matrix.....*M. proliferum*
 2. Thallus typically irregular, not necessarily catenulate or with apparent plate-like partitions between cells; cells often more elongate or variable in form.....*M. rabenhorstii*

OUR COLLECTIONS AND OBSERVATIONS

(See Figs. 1-5, and Figs. 6-7 mentioned in Discussion)

Although ours is apparently the first report of *Myzocyttium* from Alabama, this finding is not surprising, given a broad distribution of the genus (cf. Sparrow, 1960). Our study (in two counties of west-central Alabama—Tuscaloosa Co. and Choctaw Co.) focused on what we came to refer to as the *M. proliferum*/*M. megastomum* complex. Specimens—collected in late spring at locations in Northport, Tuscaloosa, and Jachin, AL (collections: WB68,70,71,133)—were obtained from *Spirogyra* occurring in modest accumulations (in shallow pond margins, stagnant creeks, ditches, etc., Figs. 1a,b) around submerged bases of cattails (*Typha* sp.) or other aquatic angiosperms. These *Myzocyttium* specimens were parasitic within *Spirogyra* vegetative cells (Figs. 2a,b,c); variously, they could exhibit features of both *M. proliferum* and *M. megastomum* (as subsequently discussed). Special emphasis has been given to the morphology of the zoosporangial discharge tube (Wildeman, 1893; Canter, 1947; Sparrow, 1960; Karling, 1981). Although *M. proliferum* is envisioned to possess a uniformly cylindrical sporangial discharge tube, the acceptance of some variation in morphology (i.e., the amount of tube swelling) is evident (cf., Martin, 1927; Sparrow, 1960; Karling, 1981); Canter (1947) thought that the somewhat swollen discharge tube of specimens recognized by Martin (1927) as *M. proliferum* might in fact be more indicative of *M. megastomum*. A distinct swelling of the discharge tube, internal to its exit from the host cell, has indeed been considered a diagnostic feature of *M. megastomum* (Wildeman, 1893; Canter, 1947). Alabama specimens exhibited discharge tubes representative of either species (Figs. 3a,b,c), but were often varyingly intermediate (Figs. 4a,b,c). It remains for a future study to decipher the extent to which the “environment” encountered inside the host-cell (e.g., the path to, and the thickness/resistance of, the host cell-wall) determines the bulge occurring in the discharge tube, as opposed to the role in this morphology played by genetics. In any case, our observations led us to question the morphological distinction of these species in our study area. Delimitation by host occurrence may also be questionable since specimens identifiable as *M. megastomum*—reported (Wildeman, 1893; Canter, 1947) in desmids—were found in *Spirogyra* (Figs. 3b,c), as were (more expectedly) forms identifiable as *M. proliferum* (Fig. 3a; 5). Sparrow’s (1960) report of *M. megastomum* from *Cladophora* was based on Martin’s (1927) report of “*M. proliferum*.”

DISCUSSION

Among organisms formerly placed in the Lagenidiaceae (see Sparrow, 1960, for traditional classification, and Dick, 2001, for review of revisions of classification), there is considered to be generic variation not only in the category of host occupied but in the position (relative to asexual reproductive structures produced) of occurrence of zoosporogenesis. *Myzocyttium* is now circumscribed (Dick, 2001) to encompass only algal parasites; these possess extra-sporangial sporogenesis, cleavage of zoospores (or completion of same) occurring in a thin, external vesicle at the distal end of a discharge tube (see interpretation of *Myzocyttium* by Pereira and Vélez, 2004, in light of Dick’s 1997 paper on Myzocytiopsidaceae). The genus *Myzocytiopsis* was established (Dick, 1997) for organisms, similar to *Myzocyttium*, which are invertebrate parasites—of nematodes, rotifers and amphipods (see Kiziewicz and Nalepa, 2008, re: amphipod parasitism)—and which exhibit intra-sporangial sporogenesis (no external vesicle produced, though a discharge tube may be present). Traditional *Lagenidium* (cf. Sparrow, 1960)—most species now dispersed between several genera (Dick, 2001)—contained organisms exhibiting both modes of zoospore development, although external development was more common; Karling (1981) noted that both modes can occur in one species, *L. oedogonii*. Zoospore formation in all genera discussed should be reinvestigated. In specimens of putative *Myzocyttium* we observed (algal parasites) zoospores completed development in an external cluster (Figs. 5,6)—probably vesiculate (based on the compact, rounded grouping at the tip of the discharge tube)—reinforcing placement in genus *Myzocyttium* (*sensu* Dick, 2001). While some authors (e.g., Glockling and Beakes, 2006) followed Dick’s (1997, 2001) recognition of *Myzocytiopsis* for *Myzocyttium*-like organisms infecting invertebrates, others (e.g., Kiziewicz and Nalepa, 2008) did not, interpreting *Myzocyttium* broadly. As stated, the genetic integrity (inclusiveness) of *Myzocyttium* requires confirmation. The kinds of host organisms invaded should be reinvestigated—not only because of our finding of *M. megastomum* in *Spirogyra* (rather than desmids),

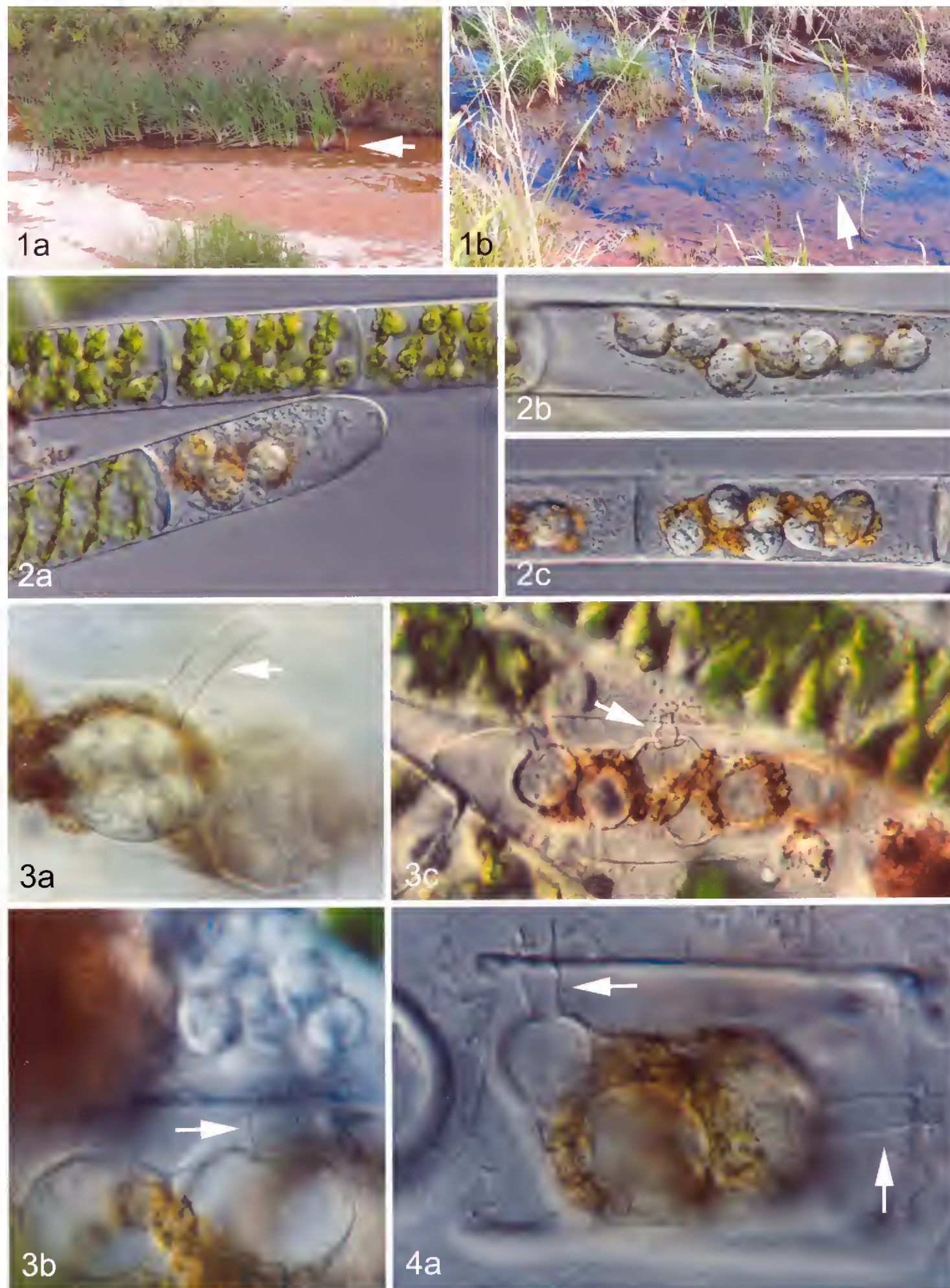
but because Cieczuga and Muszyńska (2004) reported *Myzocytiopsis microspora* (a rotifer parasite) from plant spores. Developmental studies would also prove interesting. As mentioned, cells of *Myzocyttium* are prone to be spheroidal (in contrast to the often more elongate or variable cells of *Lagenidium*). In apparent contradiction to information in Karling (1981, p. 91, last paragraph), this more spherical shape (in *Myzocyttium*) appears to “evolve” during development (Figs. 7a,b; 2b)—the ellipsoid shape of young cells of *Myzocyttium* being reminiscent of mature cells of *Lagenidium*. Again, in seeming contrast to what Karling indicated, cells of *Myzocyttium* may be distinctly constricted and appear septate or “partitioned” at an early stage (7a).

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Figures 1-7: *Myzocytium* habitat, host and specimens from Alabama. **Figs. 1a,b:** Habitat: Specimens of *Myzocytium* may be found in stagnant creeks or ditches in *Spirogyra*, accumulating around bases of cattails (1a, arrow) or other “emergent” vegetation (1b, algae present, arrow). **Figs. 2a,b,c:** Relatively mature vegetative cells of *Myzocytium* within *Spirogyra* cells: (2a) parasitizing a terminal *Spirogyra* cell; (2b,c) in intercalary cells of *Spirogyra* filament; note chain-like (2b) and more tiered (2c) morphology of the *Myzocytium* thallus. **Figs. 3a,b,c:** Zoosporangial discharge tubes: characteristic of *Myzocytium proliferum* (3a, arrow), and of *M. megastomum* (3b,c; arrows); discharged zoospores are evident (3b, above arrow and host cell-wall). **Figs. 4a,b,c:** Intermediate forms of discharge tube: Discharge tube suggestive of *M. megastomum* (4a, left arrow) and of *M. proliferum* (4a, right arrow) present on the same thallus. Cells (as vegetative cells, or converted to zoosporangia) of *Myzocytium* thallus range in size between 12 and 50 μm .

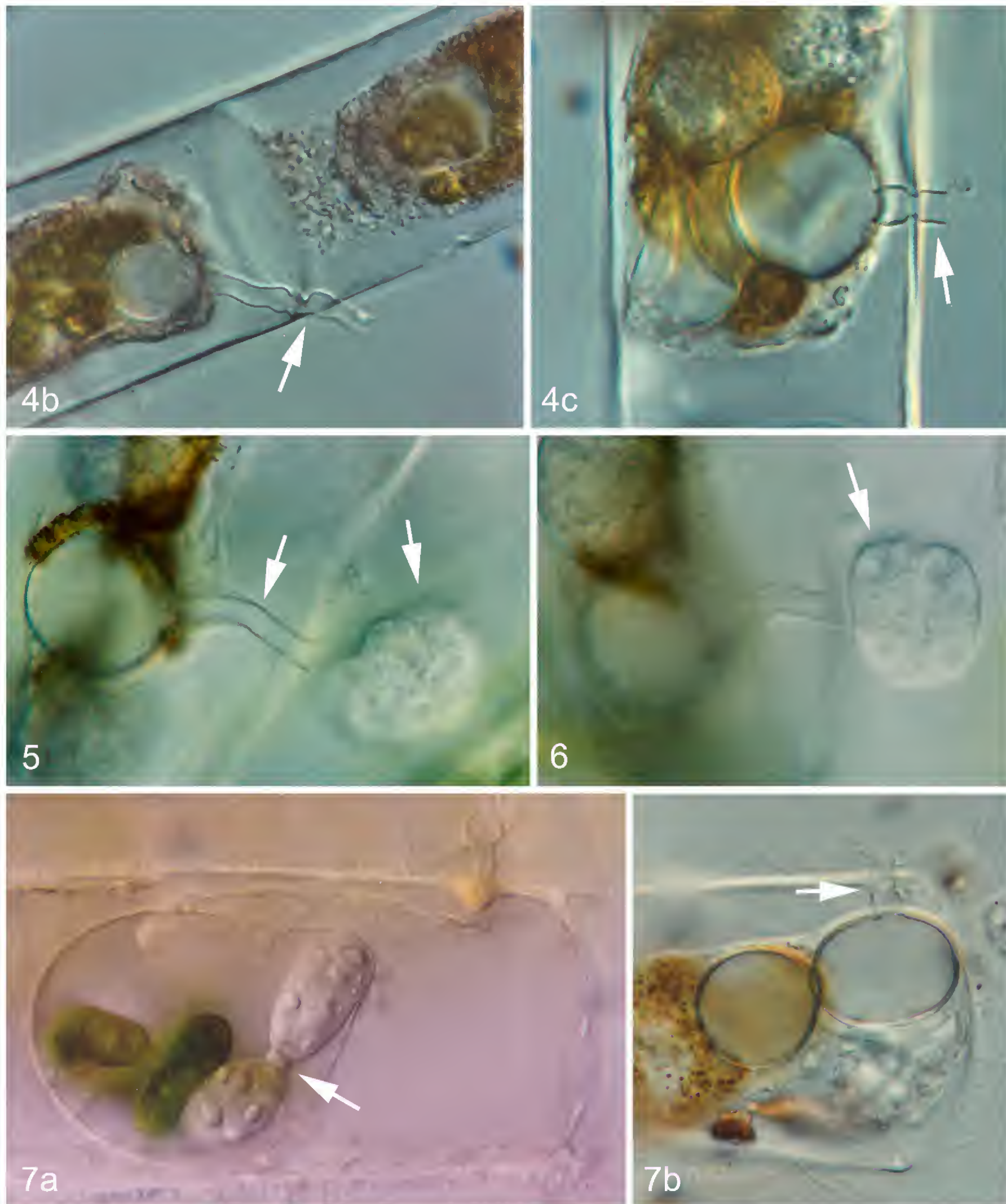


Fig. 4b: Discharge tube (arrow) resembling that of *M. proliferum*; 4c (arrow) discharge tube more like that of *M. megastomum*. **Figs. 5 and 6** (Zoospore discharge): (5) Zoospore mass (right arrow) which has been discharged from an evacuation tube (left arrow) with a morphology consistent with that of *M. proliferum*; the rounded, probably vesiculate, nature of the developing zoospore mass is evident in Fig. 6 (arrow). **Figs. 7a,b:** Developmental change in shape in cells of *Myzocyttium*: Young cells (7a) are often ellipsoid (note plate-like partition between cells, arrow); mature cells are more spherical (7b, 2b), and can develop a generally thickened wall (7b, note also old discharge tube on upper part of cell to right, arrow). Thallus cells (zoosporangia) of *Myzocyttium* are generally between 12 and 50 μm in diameter.

A survey of percent-filled and empty seeds in *Juniperus* of the western United States

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ABSTRACT

The percent-filled empty seeds for 13 *Juniperus* species in the western United States was determined by X-ray analysis. The amount of variation in % filled seeds was remarkable, varying both by year and by location, ranging from 0.0 (*J. osteosperma*, Utah) to 79.0% (*J. osteosperma*, Sedona, AZ). Of interest is the variation from 2010 to 2011, with *J. osteosperma* (Utah) having 0.0 in 2010 and 0.4% in 2011. Yet, *J. osteosperma* (Sedona, AZ) had 79.0% filled in 2010, but only 7.2% filled in 2011. Interestingly, *J. deppeana* had a similar pattern: 38.2% filled in 2010 and 0.0 % filled in 2011. But, *J. arizonica*, collected nearby, had the same % filled in 2010 and 2011. Additional studies are needed to determine if *Juniperus* has a diurnal pattern in filled seeds as common in pinyon pine. Published on-line www.phytologia.org *Phytologia* 96(2): 47-57 (April 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus arizonica*, *J. ashei*, *J. californica*, *J. coahuilensis*, *J. communis* var. *depressa*, *J. deppeana*, *J. grandis*, *J. monosperma*, *J. occidentalis*, *J. osteosperma*, *J. pinchotii*, *J. scopulorum*, *J. virginiana*, filled and empty seeds, X-ray analysis.

Juniperus is a very diverse group of taxa that are mostly dioecious (Adams, 2014). Ortiz, Arista and Talavera (1998) noted that the dioecious nature of *Juniperus* could lead to a lack of pollination. I (RPA) have noticed many cases in *Juniperus* where only the tree side facing prevailing winds produces seed cones. Ortiz, Arista and Talavera (1998) reported the incidence of filled seeds in three populations of *J. macrocarpa* Sibth. & Sm. [*J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball.] ranged from 0.04% to 65.5%, with an average of 30.7% filled seeds. In 2 of the 3 populations, the modal group was 0.0% filled seeds. They also reported on 3 populations of *J. oxycedrus* L. and found that the incidence of filled seeds ranged from 0.0% to 95%, with an average of 30.35 filled seeds. In each of the 3 populations, 0.0% filled seeds was the mode.

Houle and Babeux (1994) examined 200 seed cones (300 - 500 seeds) from each of 5 populations of *J. communis* var. *depressa* Pursh. from subarctic Quebec and found that one population had only 4% of the seeds contained embryos, whereas the other 4 populations ranged from 40 - 60% filled seeds.

Garcia et al. (2000), in an excellent study, examined seed cones and seeds of *J. communis* from 31 populations in 7 regions throughout Europe into Siberia. Filled seeds varied: South Iberian Peninsula: 0 - 40%; Central Iberian peninsula: 2 - 12%; North Iberian peninsula: 2 - 35%; Alps: 36 - 60%; Great Britain: 10 - 40%; Saian Mtns., Siberia: 75 - 80%; Northern Scandinavia: 8 - 52%. Thus, their study found a large range of filled seeds from 0 to 80%.

Douaihy et al. (2013), in a comprehensive study of *J. excelsa* from Lebanon, reported that filled seeds varied from 5% (El Njass) to 42% (Qammoua).

In a study of cone characteristics of *J. cedrus* and *J. brevifolia* (Rumeu, et al., 2009) found that 41.5% filled seeds in *J. cedrus* (with 64.6% viable) and 87.1% filled seeds in *J. brevifolia* (with 4.7% viable). The percent filled seeds for *J. brevifolia* is the highest level reported for any juniper species. *Juniperus brevifolia* is endemic to the Azores and occupies mesic sites compared to drier sites for *J. cedrus* (endemic to Canary Islands).

Fuentes and Schupp (1998) examined the incidence of filled seeds in a semi-desert species, *J. osteosperma* from North America. They questioned whether the plain titmice (*Parus inornatus*), a seed-eating bird that feeds on the seed embryos of *J. osteosperma* might select seed cones that contain filled seeds versus those with empty seeds. Table 1 shows a comparison of paired trees (adjacent) that showed either high or no predation. They found the % filled seeds to be highly significantly different between high and no predation trees. Of interest is the fact that 11 of the 17 trees had 0 to 2% filled seeds.

Table 1. Percent filled seed cones from *J. osteosperma* trees suffering high levels of seed predation by titmice and matched trees with no predation. Numbers of seed cones examined in parenthesis. Note: *J. osteosperma* averages about 1 seed/cone (Adams 2014). (from Fuentes and Schupp, 1998).

Tree Pair #	High predation, % filled (#cones)	No predation, % filled (#cones)
1	11.81 (127)	2.04 (98)
2	4.13 (121)	0.00 (105)
3	17.33 (150)	5.36 (112)
4	16.30 (92)	16.15 (161)
5	4.43 (203)	0.74 (136)
6	14.81 (243)	0.00 (130)
7	3.33 (120)	1.08 (185)
8	4.30 (93)	3.61 (166)
9	3.23 (93)	0.00 (75)
10	7.84 (153)	0.00 (172)
11	3.18 (157)	7.44 (121)
12	12.65 (253)	3.24 (432)
13	15.45 (343)	0.44 (455)
14	3.78 (291)	1.38 (289)
15	0.40 (251)	0.00 (304)
16	16.50 (618)	0.35 (282)
17	9.19 (272)	0.38 (526)
Mean (std dev)	8.74 (5.83)	2.48 (4.12) p = 0.001 ***

Recently, we (Adams and Thornburg, 2011) reported on an unusual phenomenon that some *J. arizonica* male trees produced a few seed cones among their pollen cones. However, at the time, we did not know if the seed cones borne on male tree contained filled seeds. A few seeds were examined from nearby female trees of *J. arizonica* and found to contain no embryos (empty). Before damaging the seeds from the male trees by opening them, a survey of the seed literature revealed that the US Forest Service, National Seed Laboratory, Dry Branch, GA provides a non-destructive X-ray service to determine if seeds contain an embryo. However, a general survey was needed as background for the incidence of filled seeds in *J. arizonica* and this led to a more general survey of the incidence of filled vs. empty seeds for most of the *Juniperus* species of the western United States. The purpose of this paper is to report on the results of that survey.

MATERIAL AND METHODS

Plant specimens collected:

- J. arizonica*, Adams 12505-12509, 5 normal female trees, Cottonwood, AZ, 3 Nov 2010, Adams 13178-13182, 5 normal female trees, Cottonwood, AZ, 28 Nov 2011, Adams 12510-12516, 7 'male' trees with seed cones, Cottonwood, AZ, 3 Nov 2010,
J. ashei, Adams 12500-12504, Westlake Hills, Austin, TX, 30 Oct 2010,
J. californica, Adams 13249-13253, Bodfish, CA, 14 Mar 2012, Adams 13254-13258, Victorville, CA, 14 Mar 2012, Adams 13260-13264, Bagdad, AZ, 16 Mar 2012,
J. coahuilensis, Adams 12574-12578, Alpine, TX, 20 Dec 2010 (mostly ripe, pink),
J. communis var. *depressa*, Adams 12384-12387, 6 Aug 2010,
J. deppeana var. *deppeana*, Adams 12551-12555, 14 mi se Camp Verde, AZ, 34.489386° N, 111.624069° W, Nov 2010, Adams 1384-13187, 14 mi se Camp Verde, AZ, 34.489386° N, 111.624069° W, Nov 2010,
J. grandis, Adams 12318-12322, Onyx Summit, CA, 20 Jul 2010 (seed cones green, too early to be filled?), Adams 12527-12531, Onyx Summit, CA, 5 Nov 2010 (seed cones blue, ripe, soft),
J. monsperma, Adams 12569-12573, Lake Tanglewood, Palo Duro Canyon, TX, 17 Dec 2010 (very ripe),
J. occidentalis, Adams 12343-12345, sw of Susanville, CA, 23 Jul 2010 (too early to be filled?), Adams 12476-12480, Bend, OR, 10 Oct 2010 (seed cones blue, ripe, soft),
J. osteosperma, Adams 12408-12412, Big Cottonwood Canyon, Salt Lake City, UT, 4 Sep 2010 (too early to be filled?), Adams 13188-13192, Big Cottonwood Canyon, Salt Lake City, UT, 4 Dec 2011, Adams 12323-12327, Big Bear Basin, CA, 20 Jul 2010 (too early to be filled?), Adams 12546-12550, n of Sedona, AZ, 34.491521° N, 111.690468° W, Nov 2010, Adams 13174-13177, n of Sedona, AZ, 34.491521° N, 111.690468° W, Nov 2011,
J. pinchotii, Adams 12540-12544, 15 m s of Claude, TX (Palo Duro Canyon), 14 Nov 2010 (seed cones ripe, orange, pollen shed in Sep-Oct),
J. scopulorum, Adams 12561-12565, Cimarron Canyon, NM, 2 Dec 2010 (2 yr, ripe cones),
J. virginiana, Adams 121495-12499, Lockhart, TX 30 Oct 2010.

Voucher specimens are deposited in the herbarium (BAYLU), Baylor University, Waco, TX.

X-ray analysis of the seeds was performed by the US Forest Service, National Seed Laboratory, Dry Branch, GA.

RESULTS AND DISCUSSION

The comparison between normal, female *J. arizonica* trees and the 7 'male' trees bearing a few seed cones (Table 2) shows the seeds from the normal, female trees ranged from 20 to 56% filled seeds in 2010 and 2011, with both years very similar in % filled seeds. The 7 'male' trees, with a few seed cones, varied from 0.0 (1) to 100.0% (2), but for larger numbers of seed (trees 1, 2, 10, 17), there was considerable filled seeds (42.8, 88.0, 29.4, 50.0%, Table 2). These seed from otherwise, 'male' (i.e. pollen producing) trees were just as likely filled (and presumably viable) as the normal, female trees seed.

Table 2. The % filled seeds from normal, female *J. arizonica* trees and 7 'male' trees bearing a few seed cones.

J. arizonica, 10 normal, female trees, Cottonwood, AZ, David Thornburg property.

coll. 3 Nov 2010	# cones	seeds/cone	#seeds X-rayed	% filled	
12505	50	1.22	50	28	
12506	50	1.00	50	30	
12507	50	1.11	50	34	
12508	50	1.14	50	56	
12509	50	1.00	50	24	avg = 34.4%
coll. 28 Nov 2011			#seeds X-rayed	% filled	
13178			50	21	
13179			50	39	
13180			50	49	
13181			50	20	
13182			50	38	avg = 33.4%

J. arizonica, 7 male trees, each with a few female cones, Cottonwood, AZ, David Thornburg property.

coll. 3 Nov 2010	# cones	seeds/cone	#seeds X-rayed	% filled	
12510 tree 1	13	1.08	14	42.8*	
12511 tree 2	50	1.04	50	88.0*	
12512 tree 3	1	1.00	1	100.0	
12513 tree 4	1	1.00	1	0.0	
12514 tree 8	1	1.00	1	100.0	
12515 tree 10	37	0.92	34	29.4*	
12516 tree 17	30	1.00	30	50.0*	avg. = 52.6% (for * trees)

Juniperus ashei is an abundant juniper in the Texas Edwards Plateau, ranging northward to n Arkansas and s Missouri (Adams, 2014). It has low to moderate % filled seeds at the Austin, TX population sampled, ranging from 14 to 38% (Table 3) in the 2010 sample.

Table 3. The % filled seeds for *J. ashei*.

J. ashei, Westlake Hills, Austin, TX, coll. 30 Oct 2010.

	# cones	seeds/cone	#seeds X-rayed	% filled	
12500	47	1.06	50	38	
12501	50	1.14	50	14	
12502	32	1.56	50	26	
12503	32	1.50	50	36	
12504	50	1.28	50	22	avg = 27.2%

The California juniper, *Juniperus californica*, has a large distribution in the margins of the California central valley from near Red Bluff, southward into Baja Mexico and east from s California to nw Arizona (Adams, 2014). Two populations in California showed a considerable number of filled seeds (38 to 78%, Table 4). The population from Bagdad, AZ had very high seed fill, ranging from 68 to 92% (Table 4).

Table 4. The % filled seeds for *J. californica*.*J. californica*, Bodfish, CA, coll. 14 Mar 2012

	# cones	seeds/cone	# w 2 seeds	# w 3 seeds	#seeds X-rayed	% filled	
13249	43	1.16	7	0	50	54.0	
13250	43	1.16	7	0	50	60.0	
13251	34	1.47	16	0	50	52.0	
13252	50	1.00	0	0	50	54.0	
13253	45	1.11	5	0	50	72.0	avg = 58.4%

J. californica, Victorville, CA, coll. 14 Mar 2012

	# cones	seeds/cone	# w 2 seeds	# w 3 seeds	#seeds X-rayed	% filled	
13254	40	1.25	10	0	50	64.0	
13255	24	1.63	7	8	50	68.0	
13256	29	1.52	8	7	50	38.0	
13257	27	1.56	7	8	50	68.0	
13258	25	1.72	11	7	50	78.0	avg = 63.2%

J. californica, Bagdad, AZ, coll. 16 Mar 2012

	# cones	seeds/cone	# w 2 seeds	w # 3 seeds	#seeds X-rayed	% filled	
13260	50	1.00	0	0	50	92.0	
13261	50	1.00	0	0	50	68.0	
13262	48	1.04	2	0	50	80.0	
13263	43	1.16	7	0	50	80.0	
13264	48	1.04	2	0	50	68.0	avg = 77.6%

Juniperus coahuilensis is an usual juniper with unique, pink-colored seed cones. It is common in northern Mexico, but is uncommon in the United States, found only in the trans-Pecos Texas area (Adams, 2014). As the case in all juniper species, the seed cones are prone to insect attack and the laying of eggs in the young seed cone. The samples of *J. coahuilensis* contained one tree in which all the seed cones examined (~250) were damaged by insects. The other four trees seed cones had filled seeds ranging from 10.9 to 51% (Table 5).

Table 5. The % filled seeds for *J. coahuilensis*.*J. coahuilensis*, Alpine, TX, 20 Dec. 2010, most fruit ripe, very few greenish

	# cones	seeds/cone	#seeds X-rayed	% filled	
12574	50	1.00	49	51.0	
12575	50	1.00	46	34.8	
12576*	49	1.02	0*	0.0*	
12577	50	1.00	46	10.9	
12578	50	1.00	44	18.2	avg = 22.8%

* all seeds damaged by insects. Some larvae were still inside seeds.

The common juniper, *Juniperus communis*, is a Pan-Arctic species found at northerly latitudes. *Juniperus communis* var. *depressa* is confined to North America (Adams, 2014). The population near Winnipeg, Manitoba, Canada had low seed fill (8.3 - 16%, Table 6). This is not too dissimilar from the results of Houle and Babeux (1994) who found the incidence of filled seeds in 5 populations of *J. communis* var. *depressa* were 4, 40, 53, 57, and 60%.

Table 6. The % filled for *J. communis* var. *depressa*

J. communis var. *depressa*, Winnipeg, Canada, coll. 6 Aug 2010

	# cones	seeds/cone	#seeds X-rayed	% filled
12384	50	1.98	50	16.0
12385	50	2.32	50	12.0
12386	--	--	60	8.3
12387	--	--	50	10.0
				avg = 11.6%

The alligator bark juniper, *Juniperus deppeana*, has large, woody seed cones with 2 - 4 seeds/cone. It very common in Arizona and New Mexico (Adams, 2014). Our first samples were collected in Nov., 2010 and had filled seeds ranging from 20.8 to 48.1% (Table 7). However, seeds were collected from trees at the same population in Nov., 2011 and no (0.0) filled seeds were produced that year. Conifers are well known to produce large seed crops in alternative years (cf. pinyon pine). This may be the case in *J. deppeana* or 2010 may have been a bad year for filled seeds.

Table 7. The % filled seeds for *J. deppeana*

J. deppeana, ca 14 air miles SE of Camp Verde, Az along Hwy 260, ex David Thornburg coll. **Nov 2010**.

	# cones	seeds/cone	#seeds X-rayed	% filled
12551	20	2.30	46	21.7
12552	17	3.06	53	48.1
12553	12	4.17	45	40.0
12554	20	2.55	53	60.4
12555	21	2.38	48	20.8
				avg = 38.2%

J. deppeana, **re-collection**, ca 14 air miles SE of Camp Verde, Az along Hwy 260 ex David Thornburg, coll. **28 Nov 2011**.

	# seeds X-rayed	% filled
13183	50	0.0
13184	50	0.0
13185	50	0.0
13186	50	0.0
13187	50	0.0
		avg = 0.0%

The grand juniper, *Juniperus grandis*, was previously recognized as *J. occidentalis* var. *australis*. However, recent DNA sequencing data supports its recognition as a distinct species (Adams, 2014). Two sets of seed cone samples were collected from a population at Onyx Summit, San Bernardino Mtns., CA on 20 Jul 2010 and 5 Nov 2010. Because the seed cones were smaller and green in the 20 Jul samples, it was suspected that collecting later, when the seed cones were mature, would yield seeds easier to score. The 20 Jul seeds ranged from 4.8 to 25.0% filled, whereas the 5 Nov seeds ranged from 6.0 to 24.0% filled (Table 8). So it does not appear that using younger seeds made any difference in the ability to utilize X-ray analyses to determine filled and empty seeds.

Table 8. The % filled seeds for *J. grandis* collect at Onyx Summit, CA*J. grandis*, Onyx Summit, CA coll. 20 Jul 2010 (too early?), cones green, hard

	# cones	# w 2 seeds	#seeds X-rayed	% filled
12318	--	--	88	25.0
12319	--	--	85	22.3
12320	--	--	71	5.6
12321	--	--	73	11.0
12322	--	--	84	4.8

avg = 13.7%

J. grandis, Onyx Summit, CA, re-collection, coll. 5 Nov 2010, fruit blue, ripe, soft!

	# cones	seeds/cone	#seeds X-rayed	% filled
12527	26	1.92	50	6.0
12528	35	1.43	50	20.0
12529	35	1.43	50	24.0
12530	28	1.78	50	20.0
12531	30	1.57	50	8.0

avg = 15.6%

The one-seeded juniper, *J. monosperma*, grows from west Texas into New Mexico, Colorado, and northern Arizona (Adams, 2014). A population on the rim of Palo Duro Canyon, TX was sampled. The seeds ranged from 20.0 to 77.6% filled. The 77.6% value seems unusual (Table 9).

Table 9. The % filled seeds for *J. monosperma*.*J. monosperma*, Lake Tanglewood, TX Dec. 17, 2010, very ripe

	# cones	seeds/cone	#seeds X-rayed	% filled
12569	46	1.09	48	27.0
12570	49	1.02	43	25.6
12571	48	1.04	49	77.6
12572	49	1.02	47	34.0
12573	50	1.00	50	20.0

avg = 36.8%

The western juniper, *J. occidentalis*, is the dominant juniper (and tree) in much of eastern Oregon (Adams, 2014). Seeds from 2 populations were collected in 2010. The Bend, OR seeds ranged from 0.0 to 12.0% filled (Table 10), whereas seed of two trees from SW of Susanville, CA had 0.0 and 11.8% filled.

Table 10. The % filled seeds for *J. occidentalis*.*J. occidentalis*, Bend, OR, Collected (Mark Corbet) 10 Oct 2010, ripe, blue, soft, resinous

	# cones	seeds/cone	#seeds X-rayed	% filled
12476	29	1.72	50	0.0
12477	36	1.38	50	0.0
12478	30	1.67	50	4.0
12479	40	1.25	50	12.0
12480	39	1.28	50	12.0

avg = 5.6%

J. occidentalis, sw of Susanville, CA collected 23 Jul 2010 (too early?)

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled
12343	67	0	1.00	67	0.0
12345	50	18	1.36	68	11.8

avg = 5.9%

The Utah juniper, *J. osteosperma*, is the dominant tree in many parts of Utah and Nevada and extends into northern Arizona, southern California, and western New Mexico (Adams, 2014). Seeds were collected from a small population (50 - 100 trees) growing at the mouth of the Big Cottonwood Canyon, SLC, Utah in 2010 and 2011. No filled seeds were found in 2010 and only 1 seed (in 50, = 2%) was found in 2011 (Table 11). This is an extreme situation. Seeds from a population north of Sedona, AZ were collected in 2010 and 2011. In 2010, filled seeds ranged from 54.4 to 95.5% (Table 11), compared to 2.0 to 16.0% the next year (2011, Table 11). This change from 2010 to 2011 is similar to that found for *J. deppeana* (Table 7) from nearby Camp Verde, AZ. One population was sampled from Big Bear Basin, San Bernardino Mtns., CA in 2010. Four trees were very uniform ranging from 61.5 to 66.7% filled seeds, whereas one individual (Table 11) had no filled seeds. This reinforces the idea of collecting from several tree sources to obtain viable seeds for germination.

Table 11. The % filled seeds for *J. osteosperma*.

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **4 Sep 2010** (too early?)

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled
12408	90	0	1.00	90	0.0
12409	102	1	1.01	103	0.0
12410	101	0	1.00	101	0.0
12411	102	0	1.00	102	0.0
12412	100	6	1.06	106	0.0
					avg = 0.0%

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **4 Dec 2011** ex Andy Hornbaker

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled
13188	50	0	1.00	50	2.0
13189	50	0	1.00	50	0.0
13190	50	0	1.00	50	0.0
13191	50	0	1.00	50	0.0
13192	50	0	1.00	50	0.0
					avg = 0.4%

J. osteosperma, Sedona, AZ, coll. **Nov 2010** ex David Thornburg

	# cones		seeds/cone	#seeds X-rayed	% filled
12546	50		1.00	49	89.8
12547	49		1.02	48	85.4
12548	49		1.02	44	95.5
12549	47		1.06	48	54.2
12550	47		1.06	50	70.0
					avg = 79.0%

J. osteosperma, Sedona, AZ, coll. **Nov 2011** ex David Thornburg

				#seeds X-rayed	% filled
13173				50	4.0
13174				50	2.0
13175				50	7.0
13176				50	16.0
13177				50	6.8
					avg = 7.2%

J. osteosperma, Big Bear Basin, CA, coll. **20 Jul 2010**

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled
12323	50	2	1.04	52	61.5
12324	50	12	1.24	62	64.6
12325	50	0	1.00	50	64
12326	52	2	1.04	54	66.7
12327	45	0	1.00	45	0.00
					avg = 51.4%

Recall that Fuentes and Schupp (1998) examined the incidence of filled seeds in a semi-arid species, *J. osteosperma* from Utah (Table 1, above). Their 34 trees varied from 0.0 to 16.5% filled seeds (avg. = 5.61%). So it is interesting that the Sedona, AZ, 2011 samples display a range of variation similar to their Utah samples.

The redberry juniper, *J. pinchotii*, grows in southwest Oklahoma and west Texas, and thence into northern Mexico (Adams, 2014). Samples from a population on the north side of Palo Duro Canyon, TX had very low seed fill in 2010 (0.0 to 8.0%, Table 12). Warren (2001) examined 5000 seeds per population and found 17% filled in Palo Duro Canyon (and 9.5 to 18.1% filled in 3 other locations, Table 12). It is very likely that samples from our population, in a different year might, have been quite different in % filled seeds.

Table 12. The % filled seeds for *J. pinchotii* from Palo Duro Canyon and from Warren (2001) for four populations.

J. pinchotii, Palo Duro Canyon, 15 m s of Claude, Tx, fruit ripe, coll. 14 Nov 2010.

	# cones	seeds/cone	#seeds X-rayed	% filled	
12540	47	1.06	50	0.0	
12541	46	1.09	50	8.0	
12542	50	1.00	50	6.0	
12543	48	1.04	50	0.0	
12544	29	1.72	50	0.0	avg = 2.8%
<i>J. pinchotii</i> , from Warren (2001)			# seeds	% filled	
Palo Duro Canyon			5000	17.0	
Justiceburg, TX			5000	9.5	
San Angelo, TX			5000	18.1	
Guadalupe (Salt Flat, TX)			5000	9.9	avg = 13.6%

The Rocky Mountain juniper, *J. scopulorum*, is very widely distributed in the Rocky Mountains of western North America (Adams, 2014). Analysis of seeds from Cimarron Canyon, NM revealed seeds filled ranged from 25.5 to 79.5% (Table 13).

Table 13. The % filled seeds for *J. scopulorum*.

J. scopulorum, Cimarron Canyon, NM, 2 Dec 2010, lots of 2yr ripe cones

	# cones	seeds/cone	#seeds X-rayed	% filled	
12561	42	1.19	47	42.6	
12562	45	1.11	47	40.4	
12563	37	1.35	48	29.2	
12564	40	1.25	47	25.5	
12565	47	1.06	44	79.5	avg = 43.4%

Juniperus virginiana, eastern red cedar, is a sister species to *J. scopulorum*. It grows from the eastern part of the Great Plains to the Atlantic ocean (Adams, 2014). Normally, it has lots of filled seeds and high viability. However, the Lockhart population sampled had lots of % filled seeds, ranging from 0.0 to 4.0% (Table 14). It may be that filled seeds are low in this population, or more likely, 2010 was a poor year for filled seed production. It is surprising to find such a low percent filled seeds in *J. virginiana*.

Table 14. The % filled seeds for *J. virginiana*.*J. virginiana*, Lockhart, TX coll. 30 Oct 2010.

	# cones	seeds/cone	#seeds X-rayed	% filled	
12495	42	1.19	50	2.0	
12496	39	1.28	50	0.0	
12497	39	1.31	51	0.0	
12498	38	1.32	50	4.0	
12499	30	1.70	51	0.0	avg = 1.2%

The amount of variation in % filled seeds is remarkable, varying both by year and by location. The % filled seeds for 13 *Juniperus* species from the US and Canada are shown in Table 15. These values range from 0.0 (*J. osteosperma*, Utah) to 79.0% (*J. osteosperma*, Sedona, AZ). Of interest is the variation from 2010 to 2011, with *J. osteosperma* (Utah) having 0.0 in 2010 and 0.4% in 2011. Yet, *J. osteosperma* (Sedona, AZ) had 79.0% filled in 2010, but only 7.2% filled in 2011. Interestingly, *J. deppeana* had a similar pattern: 38.2% filled in 2010 and 0.0 % filled in 2011. But, *J. arizonica*, collected nearby, had the same % filled in 2010 and 2011 (Table 15).

Additional studies are needed to determine if *Juniperus* has a diurnal pattern in filled seeds as common in pinyon pine.

Table 15. Comparison of % filled seeds for 13 *Juniperus* species in North America.

Species, location	% filled (year)
<i>J. arizonica</i> , Cottonwood, AZ	34.4 (2010), 33.4 (2011)
<i>J. ashei</i> , Westlake Hills, Austin, TX	27.2 (2010)
<i>J. californica</i> , Bodfish, CA	58.4 (2012)
<i>J. californica</i> , Victorville, CA	63.2 (2012)
<i>J. californica</i> , Bagdad, AZ	77.6 (2012)
<i>J. coahuilensis</i> , Alpine, TX	22.8 (2010)
<i>J. communis</i> var. <i>depressa</i> , Winnipeg, Can.	11.6 (2010)
<i>J. deppeana</i> , 14 miles SE Camp Verde, AZ	38.2 (2010), 0.0 (2011)
<i>J. grandis</i> , Onyx Summit, CA	13.7 (7/2010) 15.6 (11/2010)
<i>J. monosperma</i> , Lake Tanglewood, TX	36.8 (2010)
<i>J. occidentalis</i> , Bend, OR	5.6 (2010)
<i>J. occidentalis</i> , sw of Susanville, CA	5.9 (2010)
<i>J. osteosperma</i> , Big Cottonwood Canyon, UT	0.0 (2010), 0.4 (2011)
<i>J. osteosperma</i> , Sedona, AZ	79.0 (2010), 7.2 (2011)
<i>J. osteosperma</i> , Big Bear Basin, CA	51.4 (2010)
<i>J. pinchotii</i> , Palo Duro Canyon	2.8 (2010)
<i>J. pinchotii</i> , from Warren (2001)	
Palo Duro Canyon	17.0
Justiceburg, TX	9.5
San Angelo, TX	18.1
Guadalupe (Salt Flat, TX)	9.9
<i>J. scopulorum</i> , Cimarron Canyon, NM	43.4 (2010)
<i>J. virginiana</i> , Lockhart, TX	1.2 (2010)

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Morphological comparison and key to *Juniperus deltoides* and *J. oxycedrus*

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ABSTRACT

The morphologies of *J. deltoides* and *J. oxycedrus* are discussed. The species are distinguished by their leaf base shapes (deltoid, tapered), stomatal bands (not sunken, sunken), scale cone tips (protruding, absent), scale cone tip bloom (present, absent), and tree crown shape (pyramidal, rounded). A key is presented to aid in their identification. Published on-line www.phytologia.org *Phytologia* 96(2): 58-62 (April 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus deltoides*, *J. oxycedrus*, morphology, key, taxonomy.

Although recent studies (Adams, 2004; Adams et al., 2010, 2011, Adams, et al., 2005) utilizing nrDNA sequencing, RAPDs, leaf terpenoids and morphology, clearly demonstrate that *J. oxycedrus* (*sensu stricto*) is restricted to the western Mediterranean; whereas, another, morphologically similar species, *J. deltoides* R. P. Adams occupies the eastern Mediterranean region, the taxa are difficult to recognize.

Adams (2014) recognized both *J. deltoides* and *J. oxycedrus* in his monograph of *Juniperus*. At present the distributions of *J. oxycedrus* and *J. deltoides* are shown in Figs. 1 and 2. Rajcevic et al. (2013) documented *J. deltoides* in Croatia and Serbia by leaf terpenes. The exact distribution of these taxa in Italy is not known. It is likely that they are sympatric in western Italy and other places. The purpose of this paper is to present a summary of morphological differences and a key to aid their identification.

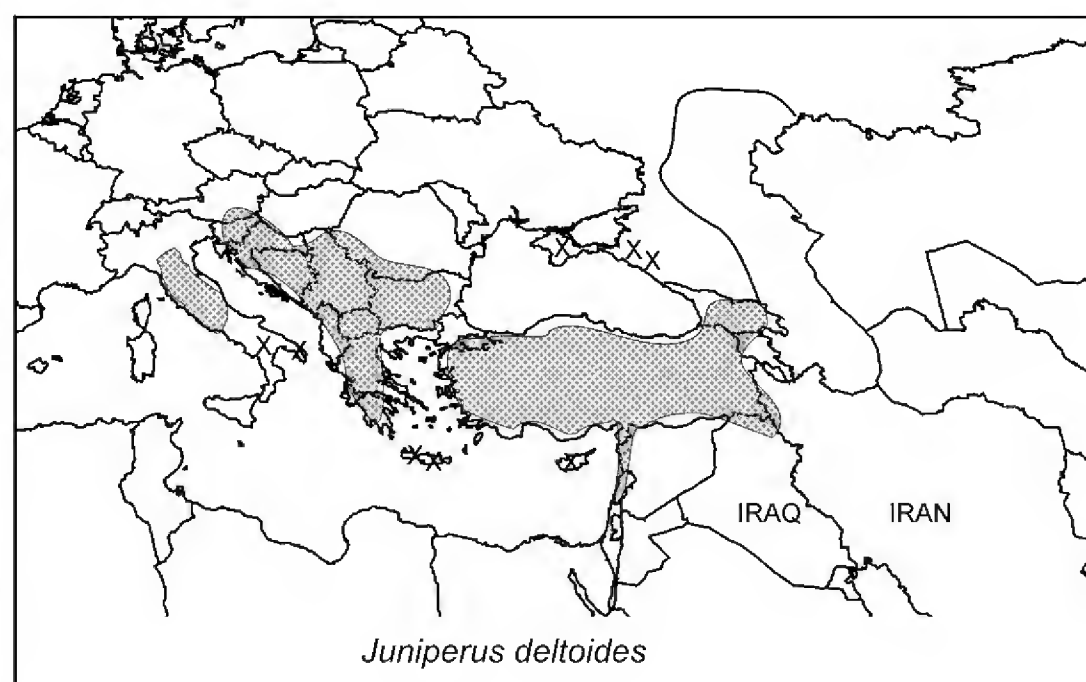
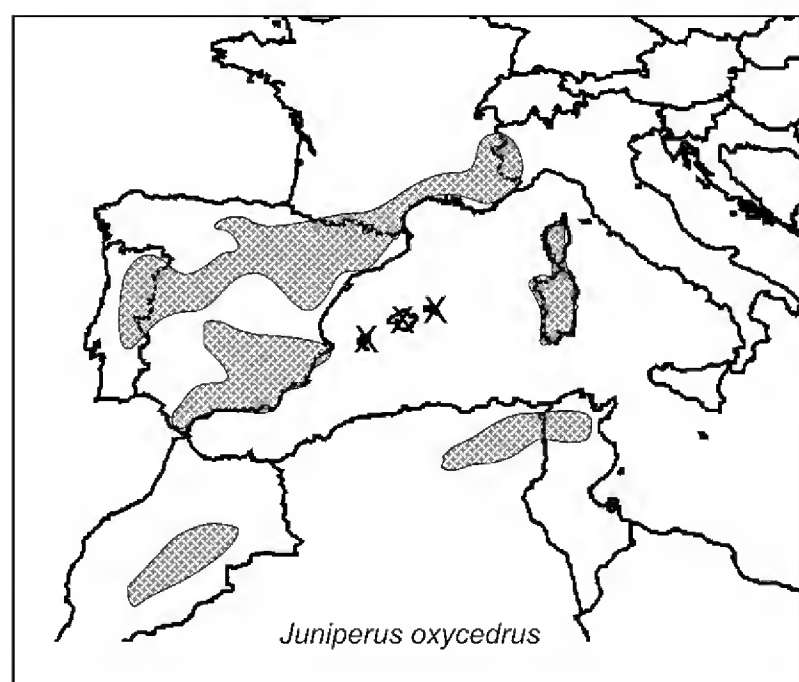


Figure 1. Distribution of *J. oxycedrus*.

Figure 2. Distribution of *J. deltoides*.

DISCUSSION

Table 1 presents a summary of morphological differences between *J. deltoides* and *J. oxycedrus* (adapted from Adams et al., 2005). The leaves of *J. oxycedrus* tend to be longer and narrower than *J. deltoides* (Table 1). A key character separating the species is the seed cone morphology. The cone scales are visible on seed cones of *J. deltoides* and the tips of the cone scales generally protrude (Fig. 3), usually

covered with a glaucous powder (bloom). In contrast, the seed cones of *J. oxycedrus* are smooth with just a hint of the 3 fused cone scales on the distal end and the cone scales are not visible on the sides of the seed cones (Fig. 4). A second key character is the shape of the leaves where the blade is joined to the sheath (base). In *J. deltoides*, the leaf sides are parallel or obtuse, giving the leaves a triangular or deltoid appearance (Fig. 5). But, in *J. oxycedrus*, the leaves taper near the base where the blade is connected to the sheath (Fig. 6).

Table 1. Morphological differences between *J. deltoides* and *J. oxycedrus* from herbarium vouchers from Morocco eastward to Turkey. protube. = protuberances (cone scale tips protruding). bloom refers the whitish-blue glaucous material on the cone-scale tips. MO = Morocco, PO = Portugal, SP = Spain, FR = France, IT (east) = eastern Italy, GR = Greece, TK = Turkey.

Character	western Mediterreanea				eastern Mediterreanea		
	MO	PO	SP	FR	IT(east)	GR	TK
leaf length(mm)	15.0	14.7	14.4	14.5	13.0	11.5	11.7
leaf width (mm)	1.7	1.43	1.28	1.70	2.10	1.80	1.80
leaf shape at base	tapered	tapered	tapered	tapered	delta	delta	delta
stomatal bands	sunken	sunken	sunken	sunken	flat	flat	flat
seed cone, distal-end							
cone scale tips	smooth	smooth	smooth	smooth	protube.	protube.	protube.
cone scale tips: bloom	none	none	none	none	bloom	bloom	bloom
tree crown shape	rounded	rounded	rounded	rounded	pyramidal	pyramidal	pyramidal

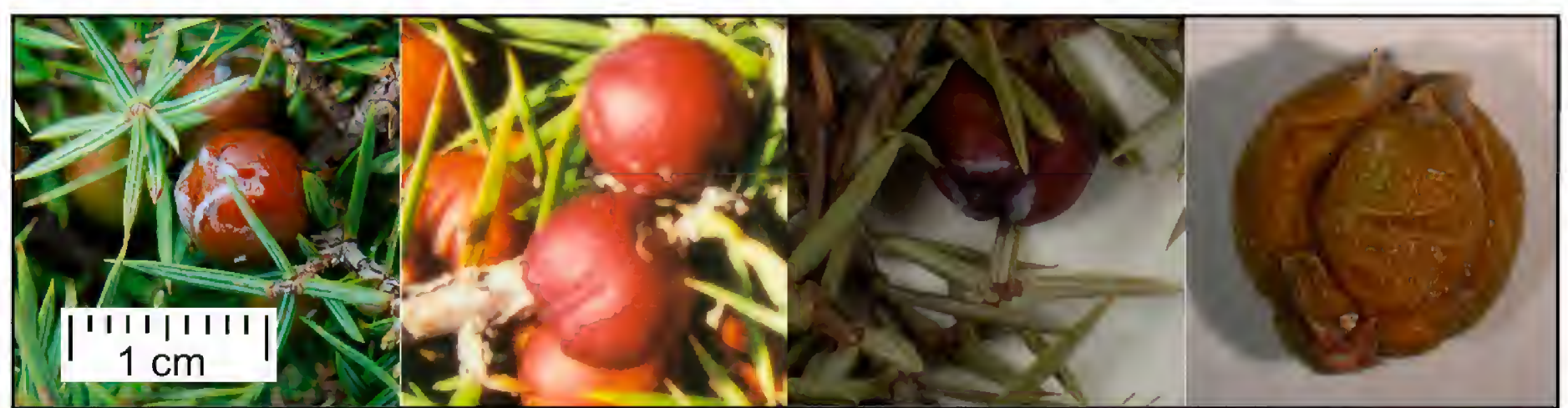


Figure 3. *J. deltoides*, seed cones (right most photo is an immature, yellow-brown seed cone with cone scales very pronounced). Notice glaucousness on cone scale tips in the left and center-right cones.



Figure 4. *J. oxycedrus*. Note the line where the 3 cones scales are fused on the distal end and the lack of protuberances on the seed cones. Cones in the right-most photo are not fully mature, thus yellow color.



Figure 5. *J. deltoides* leaf. Notice the broad (delta shaped) leaf base where the blade is joined to the sheath.



Figure 6. *J. oxycedrus* leaf. The leaves taper at the base where the leaf blade is joined to the sheath.

A third character is the position of the stomatal bands on the leaves. In *J. deltoides*, the two white, stomatal bands are not sunken, but smooth to the leaf surface (Fig. 7). In contrast, the stomatal bands of *J. oxycedrus* are sunken into the leaf (Fig. 7).

In addition the volatile leaf oil of *J. deltoides* differs from that of *J. oxycedrus* (Adams et al., 2005, Adams and Tashev, 2012). The leaf oil of *J. deltoides* is lower in α -pinene and higher in limonene compared to *J. oxycedrus*. The oil of *J. deltoides* contains several compounds not present in *J. oxycedrus*: trans-p-mentha-2,8-dien-1-ol, cis-p-mentha-2,8-dien-1-ol, cis-carveol, carvone, (2E)-decenal, ar-curcumene, α -copaen-11-ol, α -calacorene, β -calacorene and cadalene.

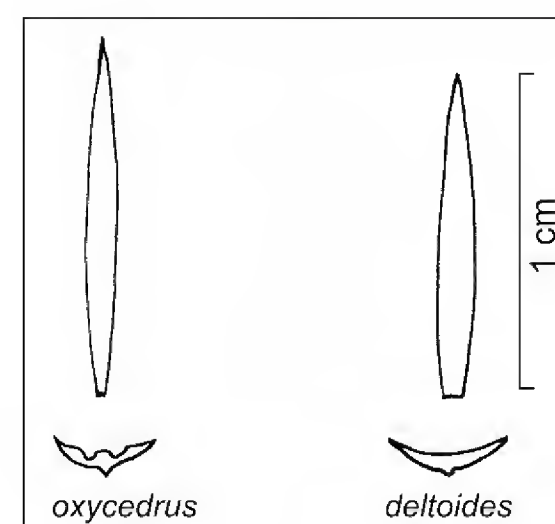


Figure 7. Leaf shapes of *J. oxycedrus* and *J. deltoides*.

The crown shape of *J. oxycedrus* is generally more rounded (Fig. 8) than the more pyramidal crowns of *J. deltoides* (Fig. 9).



Fig. 8 (left) *J. oxycedrus*, Ruidera, Spain.
Adams 9053



(right) *J. oxycedrus*, Morocco.
Adams 9406



Fig. 9 (left) Archova, Greece, *Adams 9436* (right) 30 km n. of Eskisehir, Turkey. *Adams 9430*

It might be noted that Passal (Inform. Bot. Ital. 41(1):141, 2009), published the new combination *J. oxycedrus* subsp. *deltoides* (R. P. Adams) N. G. Passal, but the combined morphology, terpenes data, and DNA sequencing clearly supports *J. oxycedrus* and *J. deltoides* as distinct lineages and species (Adams et al. 2005, Adams, 2014).

The following key is presented to aid the identification of these taxa.

Key to *J. deltoides* and *J. oxycedrus*

- 1a. Leaves narrowing at the base of attachment, stomatal bands sunken;
seed cones without bloom (glaucous) on cone scale tips,
seed cones without raised cone scale tips, seed cones globose, shrubs
and trees with round crowns; France, Spain, Portugal, Algeria, Morocco....*J. oxycedrus*
- 1b. Leaves with base of attachment nearly as wide or wider than the blade (mid-point),
stomatal bands flat to surface to scarcely sunken; seed cones without bloom
(glaucous) on cone scale tips, seed cones with raised cone scale tips,
with shrub and trees with pyramidal crown, Italy, Greece, Turkey and
eastward.....*J. deltoides*

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Comparison of leaf terpenoids and tannins in *Juniperus monosperma* from woodrat (*Neotoma stephensi*) browsed and non-browsed trees

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ABSTRACT

Neotoma stephensi is the only vertebrate herbivore to specialize on *Juniperus* that is heavily defended by terpenes and tannins. A comparison between heavily, woodrat (*Neotoma stephensi*) browsed and non-browsed *Juniperus monosperma* trees revealed that the percentage of total volatile leaf oil yields was not significantly different between browsed trees (0.53%, 2hr dist., DM-basis) and non-browsed trees (0.57%, 2hr dist., DM basis). Only one terpene, p-cymene, was significantly different between browsed and non-browsed trees. Condensed tannins did not differ significantly between browsed (3.36%) and non-browsed trees (3.02%). Woodrats, in this population, may be selecting browsed trees on convenience, proximity to their midden, and safety from predators. Published on-line www.phytologia.org *Phytologia* 96(2): 63-70 (April 1, 2014).

KEY WORDS: *Juniperus monosperma*, *Neotoma stephensi*, woodrats, browsing, terpenes, tannins, diet selection.

Seminal papers in the 1970s (Rhoades and Cates, 1976; Cates and Rhoades, 1977; Feeny, 1976) enlightened biologists that plants produce defensive compounds against herbivores. Terpenes and tannins are two types of compounds produced by juniper that are known to deter herbivores (Bernays et al. 1989; Gershenzon and Dudareva 2007). Terpenes can act as feeding deterrents (Gershenzon and Dudareva 2007) and have numerous toxic actions such as central nervous system depression, contact dermatitis, lung function impairment, liver and kidney cysts and even death (Sperling et al., 1967; Savolainen, 1978; Falk et al., 1990) as well as alter microbial fermentation (Schwartz et al. 1980 a,b, Nagy et al. 1964). Tannins readily form complexes with protein that decrease the palatability of forages (McArthur et al. 1995), reduce digestive enzyme function (Pridham, 1963), and alter microbial fermentation (Lowry et al. 1996), which can lead to decreased growth rates (Mole et al. 1993). In order to consume chemically defended plants, vertebrate herbivores' may choose plants with lower levels of defensive compounds, eat a variety of plants to avoid toxic levels of any one compound, or have efficient mechanisms to deal with defensive compounds (Freeland and Janzen 1974).

Neotoma stephensi is a small mammalian herbivore (~250g) and is the only vertebrate to specialize on juniper. Vaughan (1982) investigated the feeding behavior of *Neotoma stephensi* from several sites dominated by *Juniperus monosperma* and *J. californica*. He reported that juniper leaves accounted for over 90% of the diet in woodrats. Vaughan (1982) suggested that woodrats have fine-tuned foraging abilities to select trees with low levels of defensive chemicals as seen in other pine specialists like *Sciurus aberti* (Abert's squirrel, Snyder 1992). More recent evidence suggests that *Neotoma*

stephensi has highly efficient physiological mechanisms to effectively deal with the terpenes present in *Juniperous monosperma*, especially when compared to a sympatric generalist like *Neotoma albigula* that can only consume ~25% of its diet as juniper (Boyle and Dearing 2003; Sorenson et al. 2004; Skopec et al. 2007; Skopec and Dearing 2011; Torregrossa et al. 2011)

Considering the amount of research on the specialist woodrat (*N. stephensi*), it is surprising that we could find no publication concerning the composition of *J. monosperma* leaves from browsed trees vs. non-browsed trees. The purpose of this paper is to present new data on leaf volatile oils and condensed tannins from *J. monosperma* leaves from *N. stephensi* browsed and non-browsed trees.

MATERIALS AND METHODS

Plant material: Juniperus monosperma -

2008: Skopec 1-10, not- browsed, 11-20, browsed (= lab acc. *Adams 13867-13876*, heavily browsed; *Adams 13877-13886*, not browsed), common lava cinders, 35° 26.708' N; 111° 21.572' W, elev. 5290 ft, March, 2007, Coconino Co., AZ

2013: Adams (with A. Allgood and D. Thornburg) re-collected from the same site as Skopec, *Adams 13920-13929*, heavily browsed; *Adams 13930-13939*, not browsed, common lava cinders, 35° 26.708' N; 111° 21.572' W, elev. 5290 ft, 27 June 2013, Coconino Co., AZ

Herbarium vouchers are deposited in the herbarium, Baylor University (BAYLU).

Essential oils analysis - A portion (200 g FW) of the fresh foliage was kept cool (20°C) and in the dark, then, exhaustively steam-distilled for 24 h using a modified circulatory Clevenger-type apparatus (Adams 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C until analyzed. Steam distilled leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt. / (oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 ul of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 220°C, detector 240°C, scan time 1/sec, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

Condensed tannin analysis - Condensed tannins in air dried (48 hr, 42°C) leaves were assayed for ECT, PCT, and FCT fractions by methods described by Terrill et al. (1992). Samples were oven-dried and standards prepared from Ashe juniper as recommended by Wolfe et al. (2008).

Statistical analyses - Terpenoids (as percentage of total oil and as mg per g dry foliage weight) were compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Differences were considered significant at $P \leq 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Facing the uncertainty of chemical changes in leaf samples collected in 2008 and stored (-20°C) for 5 years, the population was re-sampled in 2013. The yields of volatile leaf oils (terpenoids) in browsed (0.53%) and non-browsed (0.57%) was not significantly different for 2008 samples, nor for 2013 samples (0.56, 0.57% ns, Table 1). Likewise, for the condensed tannins, browsed vs. non-browsed, 2007:

3.36, 3.02%, ns; 2013: 3.41, 2.91%, ns, Table 1). No differences in oils or tannins were found between the fresh (2013) and 5 yr. old samples (2008).

A detailed compositional analysis of *J. monosperma* volatile leaf oils from browsed and non-browsed trees is shown in Table 1. ANOVA of the leaf volatile oils components (% total oil basis) for browsed and non-browsed trees revealed only p-cymene was significantly different (Table 1). Analysis on a mg/g basis found no significant differences in any terpenoid between browsed and non-browsed samples (Table 1).

From an examination of *J. monosperma* trees in the field, there did not appear to be any mature trees that were completely avoided (not browsed). Although, the 10 trees collected as 'non-browsed' were clearly scarcely touched (Fig. 1) and the 10 'browsed' trees were very heavily browsed (Fig. 2). In every case, a midden was present in the browsed trees sampled in this study.



Figure 1. Non-browsed *J. monosperma* tree. The ground cover is lava cinders.



Figure 2. Heavily browsed *J. monosperma* tree. A woodrat midden is under the tree at ground level.

The lack of differences in the volatile leaf oils and yield of condensed tannins between browsed and non-browsed trees was surprising in view of the report of Vaughn (1982) about the selection of trees. However, it is instructive to compare browsing (mostly goats) on two juniper species growing in the same population. For *J. ashei*, Adams et al. (2013a) found the browsers selected for lower leaf oil yield. But, in a companion study of browsed *J. pinchotii* (in the same population with *J. ashei* in the 2013a study), Adams et al. (2013b) found no significant difference in % oil yield, nor in condensed tannins or any measure of *in vitro* digestion except for neutral detergent fiber (NDF) and acid detergent fiber (ADF). Adams et al. (2013b) hypothesized that the goats (principal browser) did not select trees on any chemical character but rather used the trees for shade and congregated at trees, then took a few bites, and over time, these 'community meeting' trees became progressively more browsed.

The severe browsing by woodrats at the Arizona *J. monosperma* site (Fig. 2) suggests that proximity to the midden may be a major factor in selecting browse trees. A few young junipers about 1 to 1.5 m tall were seen in the population and none of these appeared to have been browsed. Analysis of the oil yields and condensed tannins showed no difference from older trees, either browsed or not. It may be that the small trees do not offer the woodrats sufficient cover from predators that larger trees afford.

In the present population studied, there is very little variation in yields of leaf oils composition or the concentration of condensed tannins among the *J. monosperma* trees and, thence, scant opportunity for woodrats to select for or against leaf oils or tannins. Of course, there are thousands of chemicals in leaves, so some other chemical may be selected for (or against) such as nitrogen or fiber. Or the juniper specialists unique physiological mechanisms for metabolizing terpenes (Boyle and Dearing 2003; Sorenson et al. 2004; Skopec et al. 2007; Skopec and Dearing 2011; Torregrossa et al. 2011) may be so efficient that they don't select trees to browse on for a biochemical reasons but, instead are selecting trees based on convenience, proximity to their midden and safety from predators.

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Table 1. Comparison of leaf oils obtained from woodrat browsed and non-browsed trees of *J. monsperma*. F, signif. = F ratio and significance, P= 0.05 = *; ns = non significant, nt = not tested. p-cymene, the only compound with a significant difference, is in boldface.

	Factor tested	browsed %	non-browsed %	F, signif.	browsed mg/g DW	non-browsed mg/g DW	F, signif.
	oil yields, 2h dist. (% DW & mg/g DW)						
	March, 2008	0.53%	0.57%	3.65 ns	5.30 mg	5.74 mg	3.65 ns
	June, 2013	0.56%	0.57%	0.08 ns	5.55 mg	5.74 mg	0.08 ns
	total % condensed tannins(TCT):						
	March, 2008	3.36%	3.02%	t=1.4	P= 0.26 ns		
	June, 2013	3.41%	2.91%	t=1.5	P= 0.15 ns		
	Leaf volatile terpenoids						
KI	Compound	browsed %	non-browsed %	F sig	browsed mg/g	non-browsed mg/g DW	F sig
921	tricyclene	0.1	0.2	nt	t	t	nt
924	α -thujene	t	t	nt	t	t	nt
932	α -pinene	51.3	60.0	4.228 ns	2.80	3.51	2.091 ns
945	α -fenchene	0.2	0.2	nt	t	t	nt
946	camphene	0.3	0.3	nt	t	t	nt
961	verbenene	0.2	0.2	nt	t	t	nt
969	sabinene	0.1	0.2	nt	t	t	nt
974	β -pinene	1.1	1.2	0.109 ns	0.06	0.06	1.071 ns
988	myrcene	1.7	1.8	0.669 ns	0.09	0.10	2.096 ns
1001	δ -2-carene	0.1	0.1	nt	t	t	nt
1002	α -phellandrene	0.7	0.8	0.155 ns	0.04	0.04	0.317 ns
1008	δ -3-carene	3.0	1.9	0.214 ns	0.20	0.12	0.396 ns
1014	α -terpinene	0.1	0.1	nt	t	t	nt
1020	p-cymene	1.0	0.6	5.720 *	0.05	0.03	4.155 ns
1024	limonene	2.6	2.1	0.766 ns	0.13	0.12	0.068 ns
1025	β -phellandrene	7.5	6.3	0.588 ns	0.38	0.37	0.012 ns
1044	(E)- β -ocimene	0.2	0.1	nt	t	t	nt
1054	γ -terpinene	0.5	0.5	nt	<0.05	<0.05	nt
1065	cis-sabinene hydrate	0.1	0.1	nt	t	t	nt
1086	terpinolene	1.3	1.2	0.011 ns	0.07	0.07	0.005 ns
1098	trans-sabinene hydrate	0.2	0.2	nt	t	t	nt
1100	n-nonanal	t	t	nt	t	t	nt
1112	methyl butanoate, 3-methyl-3-butenyl-, 3-	0.5	0.4	0.694 ns	0.02	0.02	0.052 ns
1122	α -campholenal	0.2	0.1	nt	t	t	nt
1136	trans-p-menth-2-en-1-ol	0.6	0.4	1.129 ns	0.03	0.02	0.644 ns
1141	camphor	1.4	1.0	0.994 ns	0.12	0.05	2.951 ns
1144	neo-isopulegol	0.9	0.5	1.936 ns	0.05	0.03	1.468 ns
1145	camphene hydrate	t	t	nt	t	t	nt
1158	trans-pinocamphone	0.2	0.2	nt	t	t	nt
1165	borneol	0.2	0.2	nt	t	t	nt
1172	cis-pinocamphone	0.1	0.1	nt	t	t	nt
1174	terpinen-4-ol	0.5	0.5	nt	t	t	nt
1179	p-cymen-8-ol	0.2	0.1	nt	t	t	nt
1186	α -terpineol	0.2	0.5	nt	t	t	nt
1193	(4Z)-decanal	0.1	0.1	nt	t	t	nt
1195	cis-piperitol	0.2	0.2	nt	t	t	nt
1207	trans-piperitol	0.5	0.3	nt	t	t	nt
1215	trans-carveol	0.1	0.1	nt	t	t	nt
1235	trans-chrysanthenyl acetate	0.2	0.1	nt	t	t	nt
1249	piperitone	0.2	0.2	nt	t	t	nt
1258	trans-myrtanol	t	t	nt	t	t	nt
1274	pregeijerene B	2.8	2.7	0.030 ns	0.16	0.15	0.052 ns
1284	bornyl acetate	0.7	0.6	0.626 ns	0.04	0.03	1.444 ns

1289	thymol	0.4	0.2	nt	t	t	nt
1298	carvacrol	t	t	nt	t	t	nt
1315	(2E,4E)-decadienal	t	0.3	nt	t	t	nt
1396	duvalene acetate	t	t	nt	t	t	nt
1417	(E)-caryophyllene	t	t	nt	t	t	nt
1489	β -selinene	t	t	nt	t	t	nt
1498	α -selinene	t	t	nt	t	t	nt
1500	α -muurolene	t	t	nt	t	t	nt
1517	nootkatene	0.2	0.2	nt	t	t	nt
1533	trans-cadina-1,4-diene	0.1	0.1	nt	t	t	nt
1548	elemol	1.6	1.4	0.068 ns	0.08	0.08	0.008 ns
1629	eremoligenol	0.3	0.2	nt	t	t	nt
1630	γ -eudesmol	1.1	0.9	0.984 ns	0.06	0.05	0.281 ns
1640	epi- α -muurolol	t	t	nt	t	t	nt
1649	β -eudesmol	3.7	2.8	1.189 ns	0.19	0.17	0.517 ns
1652	α -eudesmol	2.7	2.2	0.715 ns	0.14	0.13	0.088 ns
1688	shyobunol	t	t	nt	t	t	nt
1746	8- α -11-elemodiol	0.6	0.5	2.538 ns	0.03	0.03	0.556 ns
1792	8- α -acetoxyelemol	1.2	1.0	0.001 ns	0.07	0.07	0.001 ns

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Taxonomy of *Hesperocyparis montana*, *H. revealiana* and *H. stephensonii*: Evidence from leaf essential oils analyses and DNA sequences

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ABSTRACT

DNA sequences and leaf essential oils were analyzed from *Hesperocyparis montana*, *H. revealiana* and *H. stephensonii*. The cpDNA sequences placed *H. stephensonii* in a clade with *H. arizonica*, *H. forbesii*, and *H. guadalupensis*, separate from the clade for *H. montana* and *H. revealiana*. The generally considered close relationship between *H. montana* and *H. revealiana*, and *H. stephensonii*, was not supported by DNA sequencing. The cpDNA sequences differ by 3 mutations between *H. montana* and *H. revealiana*. Bayesian analysis provide strong support for an *H. montana* + *H. revealiana* clade, as well as for a *H. montana* clade to the exclusion of *H. revealiana*. Support for *H. revealiana* as separate from *H. montana* is less defined. Two chemotypes were found in *H. montana*: high cedrol (28.2 - 33.7%) and low cedrol (0.02 - 0.5%). The oil of *H. montana* differs from *H. revealiana* by the presence of 2-nonanone, borneol, linalool, carvone, α -copaene, γ -muurolene, epi-cubebol, α -muurolene, γ -cadinene, endo-1-bourbonanol, α -cadinene, germacrene D-4-ol, 1-epi-cubenol, amorpho-4,9-dien-2-ol, oplophenone, oplopanonyl acetate, manoyl oxide and nezukol. The leaf oil of *H. revealiana* differs from *H. montana* by the presence of cis- and trans-p-menth-2-en-1-ols, karahanaenone, terpinen-4-yl acetate, α -terpinyl acetate, epi-zonarene, cis-muurola-4,5-diene, cis-muurola-5-en-4- α and β -ols, and α -acoreanol. The leaf oil of *H. stephensonii* was high in sabinene (9.9%) camphor (9.1%) and terpinen-4-ol (8.9%), with moderate amounts of α -pinene, limonene, β -phellandrene, α -cadinol and 2.6% iso-abienol, not found in the other taxa. The leaf oils of each of the three taxa are quite differentiated and are as dissimilar as many *Hesperocyparis* species. The differences in leaf oil compositions and DNA among *H. montana*, *H. revealiana* and *H. stephensonii* support the recognition of these species. Published on-line www.phytologia.org *Phytologia* 96(2): 71-83 (April 1, 2014). ISSN 030319430

KEY WORDS: *Hesperocyparis* (=Cupressus) *montana*, *H. revealiana*, *H. stephensonii*, volatile leaf terpenoids, cp DNA sequences, taxonomy, cedrol chemotypes.

The taxonomic position of the western hemisphere cypresses have been the source of considerable flux in the past. Commencing with a study by Little et al. (2004), utilizing DNA sequencing data, demonstrated the eastern hemisphere and western hemisphere cypresses in a clade that contained *Juniperus*, *Xanthocyparis*, *Chamaecyparis nootkatensis*. The authors noted that the western hemisphere cypresses were in need recognition as a separate genus. Little (2006) recognized the new world cypresses (*Cupressus*) as *Callitropsis*, along with the inclusion of *Chamaecyparis nootkatensis* as *Callitropsis*

nootkatensis and maintained *Cupressus* for the old world cypresses. However, using additional DNA sequence data, Adams, Bartel and Price (2009) placed the new world cypresses in a clade, distinct from *Callitropsis nootkatensis*, and a new genus was recognized for the new world cypresses, *Hesperocyparis*. Mao et al. (2010), although focusing on *Juniperus* biogeography, included several cypresses from the old and new worlds, which they treated as *Cupressus* and *Hesperocyparis* (see Fig. 2, in Mao et al., 2010). In addition, they included *Callitropsis nootkatensis* and *Xanthocyparis vietnamensis* as monotypic genera.

The most robust study to date of the phylogeny of *Hesperocyparis* (Terry, Bartel and Adams, 2012) used 12.8 kb of nrDNA and cpDNA sequence data (Fig. 1). High (1.0) posterior probabilities support were found in support for the recognition of five genera: *Hesperocyparis* (western hemisphere cypresses), *Callitropsis* (monotypic *C. nootkatensis*), *Xanthocyparis* (monotypic *X. vietnamensis*), *Cupressus* (eastern hemisphere cypresses) and *Juniperus* (used as an outgroup). Unfortunately, *H. revealiana* was not included in their study.

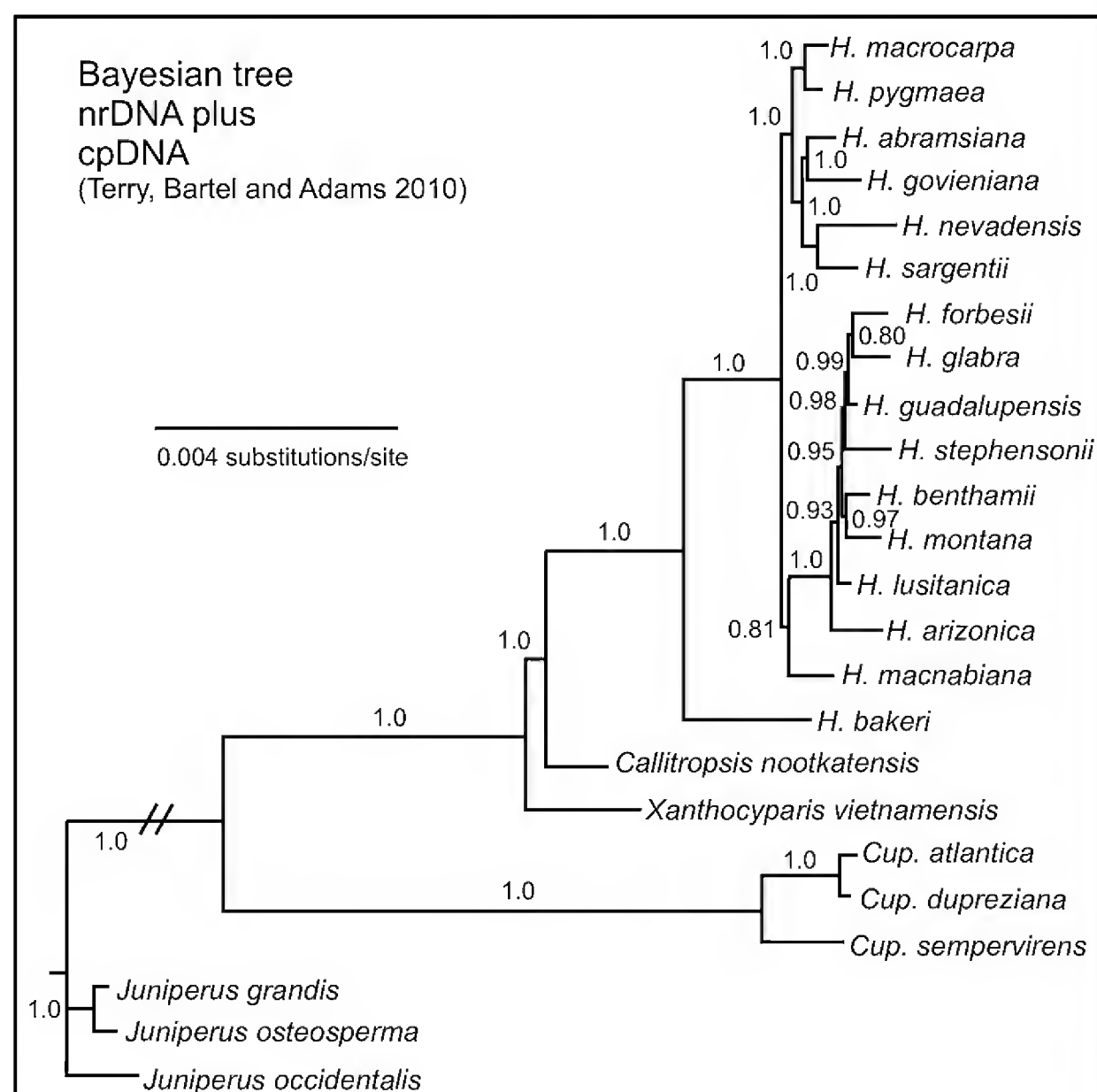


Figure 1. Bayesian tree of *Hesperocyparis*, *Callitropsis*, *Xanthocyparis*, *Cupressus* and *Juniperus*. Numbers at nodes are posterior probabilities.

Suggesting a relationship with *Cupressus* (*Hesperocyparis*) *nevadensis* and *arizonica*, Wiggins described *C. montana* in 1933, which is endemic to the Sierra de San Pedro Mártir of central Baja California of Mexico (Figure 2). Wolf also treated *C. montana* as a species in his 1948 monograph on western cypress. Although Elbert Little (1966) reduced *C. (H.) montana* to a variety of *C. arizonica*, recent authors treat *H. montana* as a species (e.g., Little 2006, Adams et al. 2009). Wolf (1948) described *C. stephensonii* from the King Creek watershed in the Cuyamaca Mountains of San Diego County (Figure 2), which he differentiated from *H. montana* by its “smooth polished, cherry-red or mahogany brown bark” versus the Mexican species and its “rough fissured, fibrous, gray or dark brown, non-exfoliating

bark.” Wolf (1948) also noted that the seed cones of *H. montana* open soon after maturity rather than retaining its seeds for years in closed (serotinous) cones.

In 1981, Silba described a new cypress variety from near El Rincón in the Sierra Juarez of northern Baja, Mexico (Figure 2) as *Cupressus arizonica* var. *revealiana*. The taxon had been previously noted by Broder (1963) and thought to be *Cupressus arizonica* var. *stephensonii* by Moran (1977). Silba (1998) differentiated the new variety by its “very thin cone scales” and smaller “distinctly dark” seeds. While Silba later elevated the variety to a subspecies rank in 2005 (*C. arizonica* subsp. *revealiana*), he contrasted it to the multi-colored bark and much larger, lighter-colored, and distinctly elongated winged seed of *C. stephensonii*. Silba (2009) eventually elevated the Sierra Juarez cypress to the specific level, *Hesperocyparis revealiana*. Farjon (2005), Eckenwalder (2009), and Debreczy and Racz (2011) treated *Cupressus* (*Hesperocyparis*) *revealiana* as conspecific with *C. (H.) stephensonii*. In addition, Damon Little (2005) in his dissertation on *Cupressus* sensu lato and *Callitropsis* did not recognize *H. revealiana* because he could find no morphological characteristics to distinguish it from *H. stephensonii*.

Bartel et al. (2003) used RAPDs to analyze various *Cupressus* (*Hesperocyparis*) species, however, they did not include *H. revealiana*. Bisbee and Maerki (2012) compared the morphology, phenology and physiology of *H. revealiana* to *H. stephensonii* and concluded that both taxa should be recognized as species. In addition, the authors included a table comparing the morphology and phenology of *H. montana*, *revealiana*, and *stephensonii*.

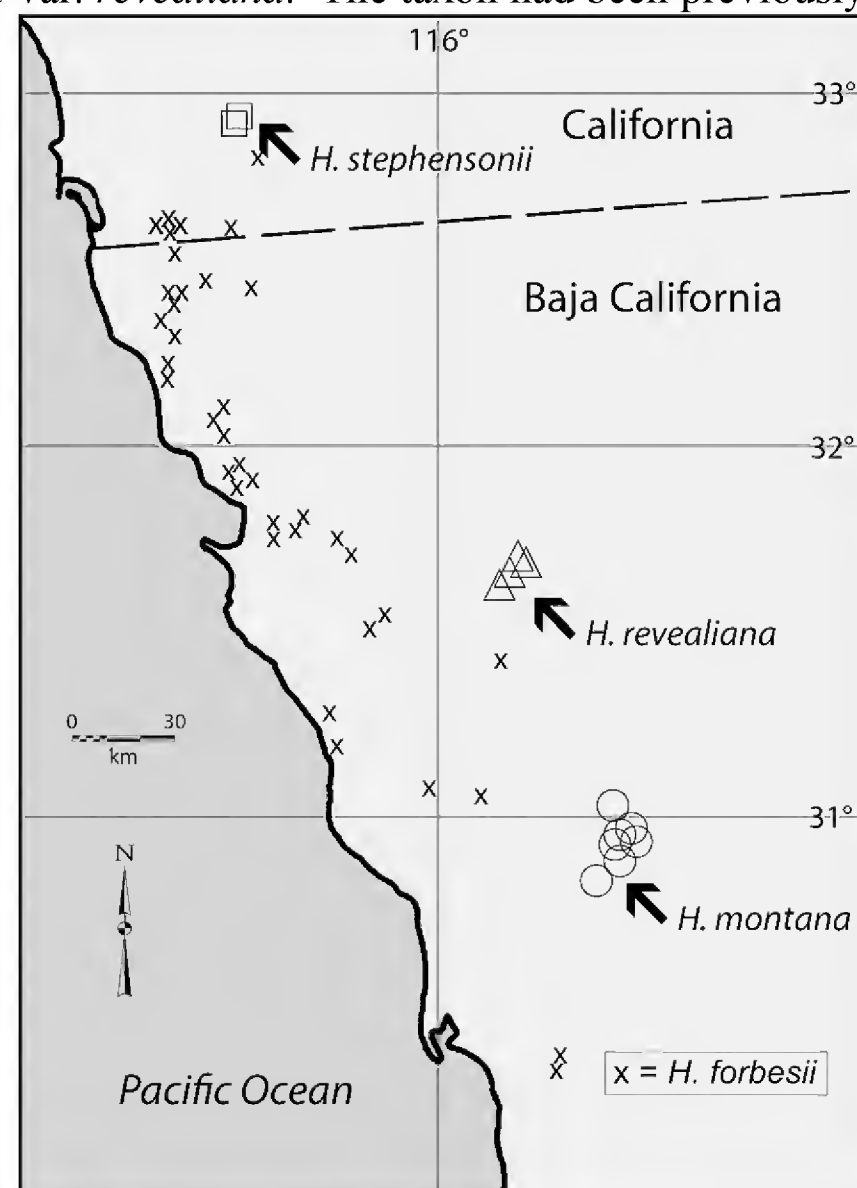


Fig. 2. General distributions of *Hesperocyparis* in Baja, MX and s CA. The Baja map portion based on Minnich (1987) and Southwestern Environmental Information Network. The California map portion based on Consortium of California herbaria records.

The purposes of this paper are to compare DNA sequences of *H. revealiana* with other *Hesperocyparis* species and to present the compositions of the leaf essential oils of *H. montana* and *H. revealiana* that, to date, have not been reported. Using these data, we address the affinity and taxonomy status of *H. revealiana*.

MATERIALS AND METHODS

Callitropsis nootkatensis, Adams 9086, Washington Park Arb., U. of Washington, Seattle, WA
Xanthocyparis vietnamensis, Adams 10142 (K. Rushforth 7745) UK, ex Vietnam
Cupressus atlantica, Adams 8429, Morocco
Cupressus dupreziana, Adams 8432, Algeria (ex Hillier Gardens)
Cupressus sempervirens, Adams 8434, Elburz Mtns., Iran (ex Hillier Gardens)
H. abramsiana, Adams 11464 (Bartel 1598a), Bonny Doon, sw of CDF fire station, CA
H. arizonica, Adams 9378 (Bartel 1580a) upper Bear Canyon, Pima Co., AZ
H. bakeri, Adams 9362, (Bartel 1572a) nw of Thousand Lake Wilderness, Shasta Co., CA

H. benthamii, Adams 8712, Pachuca, Mexico

H. forbesii, Adams 9370 (Bartel 1576a) s of O'Neal Canyon, San Diego Co., CA

H. glabra, Adams 9389 (Bartel 1585b), nw of East Verde River, Gila Co., AZ; USA

H. goveniana, Adams 11449 (Bartel 1595a), Point Lobos Ranch, Monterey Co., CA; USA

H. guadalupensis, Adams 8417, Guadalupe Island, Mexico (ex Berkeley Botanical Garden)

H. lusitanica, Adams 7072, Bussaco, Portugal (cultivated, ex Mexico)

H. macnabiana, Adams 9359 (Bartel 1569b), n of Knoxville, Napa Co., CA

H. macrocarpa, Adams 11460 (Bartel 1597b), Crocker Grove, Monterrey Co., CA

H. montana

Adams 9660-9661 (Bartel 1590a,b) collected by David R. Johnson of the Forest Service Institute of Genetics (IFG) in Placerville, California. Branches collected at IFG (Pedigree #16 Row 25 Line 12 Eddy West) from a living specimen grown from seed collected by Richard A. Minnich (UCR) on 21 July 1978,

Adams 11640(=13913) (R F Thorne et al 63552), 20 July 1988, near the end of upper Vallecito Rd., Sierra San Pedro Mártir, 2300m, Baja, Mexico, seeds sent to Huntington Botanical Gardens, 15 Feb 1990, acc 65891, Kathy Musial,

Adams 11661 (=8421), ex Inst. of Forest Genetics, Placerville, CA, ex Baja, MX, San Pedro Mártir, La Encantada meadow, 7,000', ex Berkeley Botanic Garden acc. 77.0521, Holly Forbes,

Adams 13833-13836 (Bisbee mon-1-4) cult. at Colfax, CA by Jeff Bisbee s. n., July 2001, near Botella Azul, Sierra San Pedro Mártir. Note: 13836 had unusual foliage with very short branchlets,

Adams 13840 (F. Callahan, s. n.), ex Merlin May Arb., OR, ex seed from Sierra San Pedro, Baja, CA, Mexico,

Adams 13899, (Medbury s.n.25 Feb 1996) ex. Arnold Arboretum acc. 165-96*A, wild coll. Mexico, Baja, Sierra San Pedro Mártir, 3 km from Los Llanitos Rd. at stream crossing. ca 2460m,

Adams 13901-13902, Bartel 1614, 1615, collected by R. Mitchel Beauchamp, ex Tree of Life Nursery, Orange Co., CA.

H. nevadensis, Adams 9367 (Bartel 1574b), Greenhorn Mountains, Kern Co., CA,

H. pygmaea, Adams 11489 (Bartel 1693a), Casper Little Lake, Mendocino Co., CA,

H. revealiana,

Adams 13837-13839 (Bisbee rev-1-3) cult. at Colfax, CA, Jeff Bisbee s. n., July 2004, El Rincon, Baja, Mexico. Note: 13838 had only juvenile leaves (neoteny),

Adams 13848 Bartel 1613, ex Greg Abbot s. n. 1992, foothills of Sierra de Juarez near village of Santa Catarina, Baja CA, MX, ex San Diego Zoo Safari Park, Escondido, CA,

H. sargentii, Adams 9348 (Bartel 1564c), Cuesta Ridge, San Luis Obispo Co., CA,

H. stephensonii, Adams 9376 (Bartel 1579a), Cuyamaca Rancho State Park, San Diego Co., CA,

H. stephensonii, Adams 12623-12628 (Callahan 1-6), King Creek, San Diego Co., CA

Juniperus grandis, Terry 115, Mono Co., CA,

Juniperus occidentalis, Terry 128, Baker Co., OR,

Juniperus osteosperma, Terry 058, Garfield Co., UT.

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Oils from each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007, for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

DNA extraction, PCR amplification, sequencing and data analyses

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. For primers and PCR amplifications for nrDNA (ITS) primers see Adams, Bartel and Price (2009). The trnS-trnG, trnC-trnD, trnD-trnT, and psbD/trnT intergenic spacers and the trnG intron were amplified according to Terry, Bartel and Adams, 2012.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com).

Phylogenetic analysis - A total of 4,130 bp of unambiguously aligned sequence from all species of *Hesperocyparis*, including four accessions of *H. montana* var. *montana* and two accessions of *H. montana* var. *reveliana*, was included in this study. All sequences are from chloroplast noncoding regions, including 3489 bp from four intergenic spacers (*trnS-trnG*, *trnC-trnD*, *trnD-trnT*, and *psbD-trnT*) and 641 bp from one intron (*trnG* intron). Sequence alignments were performed using ClustalW (Thompson et al. 1994; Kyoto University Bioinformatics Center, Kyoto, Japan) and refined manually using Seq-AL v.2.0a9 (Rambaut 2002). Gaps shared by two or more taxa were scored as binary characters using simple indel coding (Simmons and Ochoterena 2000) implemented in SeqState v.1.4.1 (Muller 2005, 2006). All nucleotides were included in the final alignment excluding 101 positions within the *trnS-trnG* IGS that could not be aligned unambiguously.

Bayesian analyses were performed using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Best-fit evolutionary models and analysis settings are described in Terry et al. (2012), except a burnin fraction of 0.25 was enforced, resulting in the first 1250 of 5000 trees being discarded, and the remaining trees (3750) pooled to construct the posterior distribution of the phylogeny. A 50% majority-rule consensus tree was generated from the pooled trees using the “contype=halfcompat” command. Convergence was confirmed and effective sample sizes monitored using Tracer 1.5 (Rambaut and Drummond 2007).

RESULTS AND DISCUSSION

There are no reports on the volatile leaf oils of *H. montana* or *H. reveliana*. No detailed report on the volatile leaf oil of *H. stephensonii* was found in the literature, except Cool, Jiang and Zavarin (1994) reported that *C. (H.) stephensonii* contained karahanaenone in the leaf oil. Senter, Zavarin and Zedler (1975) reported carvacrol (78%) was the major component of the heartwood oil of *C. (H.) stephensonii*.

The yields of volatile leaf oils of *H. montana* varied considerably (0.5, 0.06, 0.7, 1.2, 1.5, 0.9, 1.2, 1.1%). The *H. montana* 13834 had almost no oil (0.06%), but its oil composition was not unusual. That individual appears to have a gene almost completely blocking the mono-, sesqui- and di-terpenoids pathway. The eight *H. montana* individuals displayed two chemotypes: high cedrol and low cedrol (Table 1). Four samples (11640, 11661, 13840, 13899) were high in cedrol (28.2 to 33.7%). Six samples (13833-13836, 13901-13902) had only trace amounts (0.02, 0.05, 0.1, 0.05, 0.05, 0.5%) of cedrol. Cedrol and related compounds (Table 1, italics) are characteristic components (Adams 1991) of cedarwood oils

(eg. heartwood oils of *Juniperus* and *Cupressus*, but not *Hesperocyparis* heartwood oil, as far as known). However, occasionally in *Juniperus* leaf oils, one finds trees with considerable amounts of cedrol and associated heartwood oil components (cf. α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc., see Adams et al., 2013). Cedrol is a major component in the leaf oils of *J. excelsa*, 25.4 - 29.3% (Adams et al., 2013), *J. polycarpus*, 30.3% and *J. seravschanica*, 13.8 - 22.7% (Adams and Hojjati, 2013). In the Cupressaceae, the leaf and wood essential oils also appear to be generally distinct. However, Adams, et al. (1997) reported cedrol in several *Cupressus* (*Hesperocyparis*) leaf oils: 0.5 - 0.7%, *C. lusitanica*; 0.2% *C. lindleyi*; and 0.1% *C. glabra* (mis-reported as *C. arizonica*). Adams and Bartel (2009) found 0.1 - 0.2% cedrol in *H. goveniana*. Adams et al. (2010) reported 1.2% cedrol in *H. glabra*, but no cedrol in any population of *H. arizonica*. If one corrects for the 'cedarwood oil' components in the high cedrol *H. montana*, then the oil is similar to the low cedrol type (Table 1). The oil of *H. montana* differs from *H. revealiana* by the presence of 2-nonanone, borneol, linalool, carvone, α -copaene, γ -muurolene, epi-cubebol, α -muurolene, γ -cadinene, endo-1-bourbonanol, α -cadinene, germacrene D-4-ol, 1-epi-cubenol, amorpho-4,9-dien-2-ol, oplophenone, oplopanonyl acetate, manoyl oxide and nezukol (Table 1).

The leaf oil of *H. revealiana* differs from *H. montana* by the presence of cis- and trans-p-menth-2-en-1-ols, karahanaenone, terpinen-4-yl acetate, α -terpinyl acetate, epi-zonarene, cis-muurola-4,5-diene, cis-muurola-5-en-4- α and β -ols, and α -acorenil (Table 1). In addition, the oils differently quantitatively for many compounds (sabinene, γ -terpinene, β -phellandrene, terpinolene, camphor, umbellulone, terpinen-4-ol and trans-muurola-3,5-diene, Table 1). Overall, the number of differences between *H. montana* and *H. revealiana* is comparable to differences between recognized *Hesperocyparis* species.

The leaf oil of *H. stephensonii* was high in sabinene (9.9%) camphor (9.1%) and terpinen-4-ol (8.9%), with moderate amounts of α -pinene, limonene, β -phellandrene, α -cadinol and 2.6% iso-abienol, not found in the other taxa.

The putative hybrids (see nrDNA below), *H. montana* 11661 and 13899 did not contain compounds typical of *H. revealiana* (Table 1). Thus, the volatile leaf oils offer no support that *H. montana* 11661 and 13899 are hybrids as suggested by nrDNA.

Sequencing revealed that *H. montana* and *H. revealiana* are extremely closely related (Table 2). Eight potentially informative nrDNA (ITS) substitutions were found, but upon closer inspection it appears more likely that this is a case of incomplete lineage sorting (Syring et al., 2007) in *Hesperocyparis*. Two of the *H. montana* samples (Table 2) contained 7 complementary bases found in both *montana* and *revealiana* suggesting that these individuals are hybrids. However, the terpenoids clearly indicate that they are not hybrids. In addition, a search of GenBank revealed that, for nrDNA data, in nearly every instance where there are 2 or more accessions of a *Hesperocyparis* species, the nrDNA sequences differ by from 4 to 12 bases within the species. This well illustrates the variability of nrDNA sequences in *Hesperocyparis* and may be a consequence of incomplete lineage sorting. Thus, nrDNA data were not further utilized in this study.

The cpDNA markers exhibited very little variation between *H. montana* and *H. revealiana* (Table 2). No variation was found in psaA/ycf3 and trnT-trnTD. Only 2 SNPs were found in psbD/trnT and trnC-trnD, and these are single nucleotide differences. The trnS-trnG plus trnG intron (1661 bp) proved to be of use with 3 SNPs (1 informative) and 2 indels (both informative).

Bayesian tree analysis based on cpDNA revealed that *H. montana* and *H. revealiana* are in a well-supported clade sister to a clade containing *H. glabra*, *H. forbesii*, *H. arizonica*, *H. guadalupensis* and *H. stephensonii* (Fig. 3). The *H. montana* samples form a well-supported clade, but the two accessions of

H. revealiana are not supported as monophyletic. In contrast, this was not seen in the leaf essential oils data, where the oils were found to be relatively uniform for the four accessions of *H. revealiana*.
Table 2. Comparison of information content for primers used in this study among the 4 *H. montana* and 2 *H. revealiana* accessions examined. MEs = mutational events = # SNPs + # indels. * nrDNA was not useful due to incomplete lineage sorting.

gene region	length	# SNPs	infor. SNPs	# indels	infor. indels	infor. MEs
nrDNA (ITS)	1271	12	8	0	0	8*
montana 11661	YSYMKYYG					
montana 13899	YSYMKYYC					
montana 13840	CCTCGCTC					
montana 13838	CCTCGCTG					
revealiana 13836	TGCATTCTG					
revealiana 13837	TGCATTCTG					
cpDNA						
trnS-trnG + trnG intron	1661	3	1	2	2	3
psaA/ycf3	818	0	0	0	0	0
psbD/trnT	927	2	0	0	0	0
trnC-trnD	787	2	0	0	0	0
trnT-trnTD	721	0	0	0	0	0

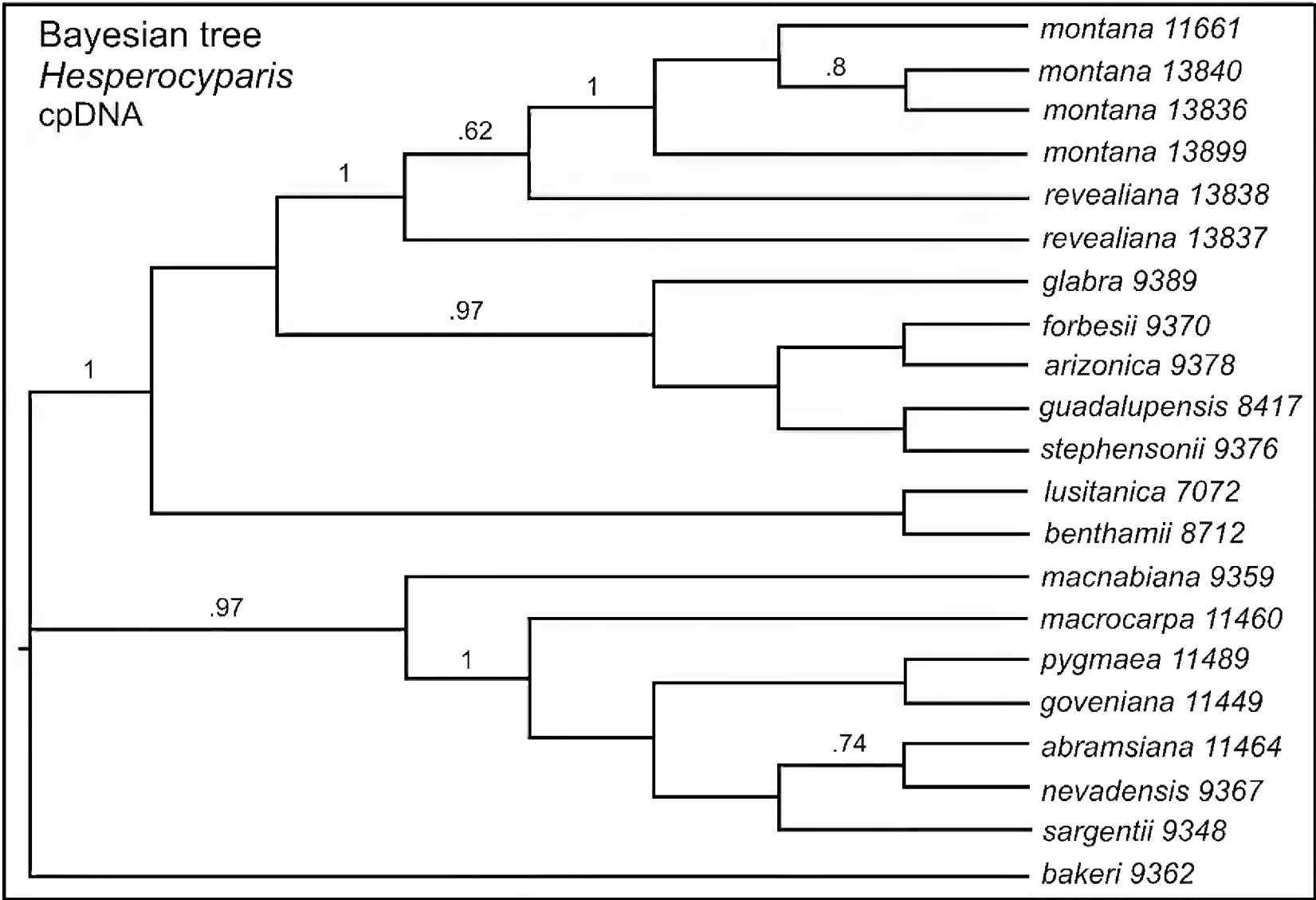


Figure 3. Bayesian tree of *Hesperocyparis* using cpDNA with *H. bakeri* as an outgroup. Numbers at nodes are posterior probabilities (PP). PP less than 0.6 are not shown.

CONCLUSIONS

The branchlets of the samples of *H. montana* and *H. revealiana* were quite variable in terms of branching angle, color, branchlet width and especially whether the cones open or remain closed on the trees (Fig. 4). The leaf oils of each of the three taxa are quite differentiated and are as dissimilar as many *Hesperocyparis* species. The differences in leaf oil compositions and DNA among *H. montana*, *H. revealiana* and *H. stephensonii* support the recognition of these species.

The cpDNA sequencing placed *H. stephensonii* in a clade with *H. arizonica*, *H. forbesii*, and *H. guadalupensis*, separate from the clade for *H. montana* and *H. revealiana*. The generally considered close relationship between *H. montana* and *H. revealiana*, and *H. stephensonii*, was not supported by DNA sequencing. The cpDNA differs by 3 mutations, and the Bayesian analysis provide strong support for an *H. montana* + *H. revealiana* clade, as well as for a *H. montana* clade to the exclusion of *H. revealiana*. Support for *H. revealiana* separate from *H. montana* is less defined. Overall, it appears reasonable to continue the recognition of *H. montana*, *H. revealiana* and *H. stephensonii*.

Figure 4. (left) *H. montana*, with cones opened on tree.

Figure 4. (right) *H. revealiana*, with cones closed on tree.

Photos by Jeff Bisbee.



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Table 1. Leaf essential oil compositions for *H. stephensonii* (*stephen*), *H. revealiana* (*reveal*) and putative hybrids of *H. montana* 11661 and 13899 that show no complementation of compounds typical of *H. revealiana*. Also shown are composite oils of *H. montana*: (*mont*) hi cedrol(11640, 11661, 13840, 13899), lo-cedrol (13833-36, 13901-2). Components typical of wood oil (in *Juniperus* and *Cupressus*) are in italics. Components that separate the taxa are in boldface. .

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> <i>11661</i>	<i>mont</i> <i>13899</i>	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
846	(E)-2-hexenal	0.3	0.9	0.4	0.2	0.3	0.3
921	tricyclene	0.2	0.5	0.4	0.1	0.5	0.9
924	α -thujene	0.6	0.5	t	-	t	0.1
932	α-pinene	6.0	12.4	5.8	3.4	3.7	13.4
945	α -fenchene	-	t	-	-	-	0.3
946	camphene	0.5	1.1	0.5	0.1	0.5	1.1
953	thuja-2,4-diene	-	t	-	-	t	0.1
969	sabinene	9.9	4.9	0.1	t	0.1	0.4
974	β -pinene	0.2	0.4	0.5	0.3	0.3	0.9
988	myrcene	2.5	3.2	0.5	0.2	0.4	1.2
1002	α -phellandrene	0.1	0.3	t	t	t	t
1008	δ -3-carene	0.7	0.4	t	t	t	3.3
1014	α-terpinene	2.0	1.7	t	t	t	t
1020	p-cymene	0.6	0.9	t	t	t	0.5
1024	limonene	4.8	12.1	3.9	2.3	4.4	8.1
1025	β-phellandrene	4.8	12.1	2.6	1.5	2.9	5.4
1038	2-heptyl acetate	-	-	t	t	0.1	0.5
1054	γ-terpinene	3.5	2.5	0.1	t	0.1	0.3
1065	cis-sabinene hydrate	0.8	0.3	t	t	t	t
1086	terpinolene	1.9	2.5	0.3	0.1	0.3	0.8
1095	trans-sabinene hydrate	0.6	-	-	-	-	-
1087	2-nonanone	-	-	-	t	0.3	0.4
1099	linalool	0.5	-	-	-	-	0.3
1100	n-nonanal	0.2	0.1	t	t	t	0.4
1118	cis-p-menth-2-en-1-ol	0.6	0.5	-	t	-	-
1122	α -campholenal	-	-	t	0.1	t	0.4
1135	trans-pinocarveol	-	-	0.1	t	t	0.5
1136	trans-p-menth-2-en-1-ol	t	0.4	-	t	-	-
1141	camphor	9.1	11.2	0.9	0.8	3.0	7.3
1145	camphene hydrate	0.5	0.5	0.2	t	0.2	0.4
1148	citronellal	0.2	0.2	-	-	-	-
1154	karahanaenone	1.6	1.7	-	-	-	-
1165	borneol	-	-	0.3	0.1	0.2	0.9
1167	umbellulone	2.8	7.0	0.1	0.2	0.2	1.2
1174	terpinen-4-ol	8.9	6.0	0.2	0.1	0.2	0.9
1179	p-cymen-8-ol	0.2	0.4	-	t	-	0.3
1186	α -terpineol	0.7	0.6	0.1	0.1	0.1	0.3
1193	(4Z)-decenal	-	0.2	-	-	-	-
1194	myrtenol	0.1	-	-	-	-	0.3
1195	cis-piperitol	0.1	0.2	-	t	-	-
1204	verbenone	-	-	t	-	-	0.3
1207	trans-piperitol	0.3	0.2	-	t	-	-
1215	trans-carveol	-	0.2	t	0.1	-	0.3

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> <i>11661</i>	<i>mont</i> <i>13899</i>	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
1223	citronellol	2.1	1.5	t	0.1	0.2	0.7
1232	thymol, methyl ether	-	0.2	-	-	-	-
1239	carvone	-	-	t	0.2	0.1	0.4
1241	carvacrol, methyl ether	-	0.1	-	-	-	-
1249	piperitone	0.1	0.1	-	-	-	-
1255	(4Z)-decen-1-ol	-	0.3	-	-	-	-
1284	bornyl acetate	0.2	0.7	3.3	t	1.6	4.3
1299	terpinen-4-yl acetate	-	0.6	-	-	-	-
1325	p-mentha-1,4-dien-7-ol	0.2	-	-	-	-	-
1346	α-terpinyl acetate	0.3	1.6	-	t	-	-
1345	α -cubebene	-	-	-	-	-	0.3
1374	α-copaene	0.2	-	0.3	0.9	0.8	1.2
1410	α -cedrene	-	-	0.8	0.8	0.8	-
1413	β -funebrene	-	-	1.5	1.5	1.7	-
1419	β -cedrene	-	-	0.9	1.0	1.1	-
1451	trans-muurolo-3,5-diene	-	1.8	-	0.2	-	0.2
1452	α -humulene	0.3	-	0.3	0.4	0.3	0.2
1461	cis-cadina-1(6),4-diene	-	-	0.3	0.5	0.3	0.2
1465	cis-muurolo-4,5-diene	-	4.1	-	-	-	-
1471	dauca-3,5-diene	-	-	-	-	-	0.2
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	0.3
1478	γ-muurolene	0.4	-	0.4	0.8	0.7	1.1
1493	trans-muurolo-4(14),5-diene	-	-	-	-	-	0.3
1493	epi-cubebol	0.5	-	0.5	1.1	1.0	0.7
1500	α-muurolene	0.8	-	0.7	1.5	1.3	1.9
1501	epi-zonarene	-	1.7	-	-	-	-
1513	γ-cadinene	2.0	-	2.3	5.6	5.1	6.2
1518	endo-1-bourbonanol	1.2	-	1.3	1.3	0.8	1.3
1521	trans-calamenene	-	0.2	-	-	-	-
1522	δ-cadinene	2.9	0.3	2.9	4.5	3.7	4.6
1537	trans-cadina-1,4-diene	-	-	-	-	-	0.4
1537	α-cadinene	0.2	-	0.2	0.5	0.5	0.7
1544	α -calacorene	-	-	t	0.3	0.3	0.5
1550	cis-muurolo-5-en-4-β-ol	-	0.3	-	-	-	-
1559	cis-muurolo-5-en-4-α-ol	-	0.3	-	-	-	-
1561	(E)-nerolidol	-	0.1	-	-	-	-
1561	germacrene D-4-ol	2.4	-	2.8	2.3	2.7	1.4
1564	β -calacorene	-	-	-	-	-	0.3
1589	allo-cedrol	-	-	1.1	0.8	0.9	-
1600	cedrol	-	-	33.7	29.8	31.4	0.1
1607	β -oplopenone	0.5	-	0.3	0.6	0.9	-
1618	epi-cedrol	-	-	-	-	0.5	-
1618	1,10-di-epi-cubenol	-	-	-	-	-	0.3
1627	1-epi-cubenol	0.4	-	0.4	1.2	1.2	1.2
1632	α-acorenol	-	0.2	-	-	-	-
1638	epi-α-cadinol	1.2	0.1	1.3	2.6	1.9	2.0
1638	epi-α-muurolol	1.2	0.1	1.2	2.6	1.8	2.0
1644	α -muurolol	0.5	-	0.4	0.8	0.7	1.0

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> <i>11661</i>	<i>mont</i> <i>13899</i>	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
1652	α-cadinol	3.5	0.7	2.8	5.5	3.6	3.8
1675	cadalene	-	-	-	-	-	0.5
1688	cis-14-nor-murol-5-en-4-one	-	0.3	-	-	-	-
1689	terpenoid, <u>43</u> ,167,218,236	0.5	-	-	-	-	0.9
1699	epi-nootkatol	0.1	-	-	-	-	-
1700	amorpha-4,9-dien-2-ol	0.1	-	0.4	0.6	0.5	0.4
1724	(Z)-nuciferol	-	-	-	-	0.3	-
1739	oplopinone	0.1	-	0.3	0.8	0.4	0.4
<i>1767</i>	<i>cedryl acetate</i>	-	-	<i>0.1</i>	<i>0.4</i>	<i>0.1</i>	-
1887	oplopanonyl acetate	0.3	-	0.1	-	0.6	0.4
1966	isophyllocladene	-	0.1	-	-	-	-
1987	manoyl oxide	0.4	-	1.2	0.3	0.6	0.3
2009	13-epi-manoyl oxide	-	-	0.3	t	0.1	-
2055	abietatriene	0.2	t	-	-	-	-
2105	iso-abienol	2.6	-	-	-	-	-
2132	nezukol	-	-	10.8	12.0	9.7	2.6
2209	phyllocladanol	1.8	0.1	0.1	0.2	0.9	0.2
2282	sempervirol	1.1	0.1	1.1	0.2	0.5	t
2314	trans-totarol	0.8	0.1	0.9	0.1	0.6	t
2331	trans-ferruginol	0.5	0.1	0.2	0.3	0.1	t

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Snowpack-forced browsing by deer onto Scots Pine (*Pinus sylvestris*): Selection of low volatile leaf terpenoid concentrations for browsing

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ABSTRACT

A comparison between heavily and lightly browsed Scots pine (*Pinus sylvestris*) trees revealed that the yield of volatile leaf oil and tree height were highly significantly different. Higher volatile oil yield was positively correlated with lightly browsed trees, and lower oil yield correlated with heavily browsed trees. Tree height was negatively correlated with terpenoid yield, indicating younger trees produced more oil, perhaps as a defense mechanisms. Methanol extractables were significantly higher in highly browsed trees, suggesting that sugars and carbohydrates might be attractive to browsing deer. Hexane extractables were not significantly different. Published on-line www.phytologia.org *Phytologia* 96(2): 84-88 (April 1, 2014).

KEY WORDS: *Juniperus pinchotii*, goats, deer, browsing, terpenes, fiber, condensed tannins, digestibility, diet selection.

Deer browsing of conifers is a common phenomenon. Two of the best documented cases are *Chamaecyparis nootkatensis* and *Thuja plicata* (Vourc'h, Russell and Martin, 2002). They found the concentrations of mono- sesqui-, and di-terpene to be high in un-browsed *C. nootkatensis* and low in browsed genets in a plot at Cowichan Lake Research Station. For *Thuja plicata*, they found monoterpenes higher in lightly-browsed trees but di-terpene concentrations were not significantly different (*T. plicata* oil contains almost no sesquiterpenes). Schwartz et al. (1980a) observed deer browsing on *Juniperus scopulorum* when deep winter snows in northern Colorado prevented access to their regular browse species. They conducted feeding trials on confined deer using foliage of *J. deppeana*, *J. monosperma* and *J. scopulorum* and found the consumption of juniper foliage varied inversely with the total oil yields among these three species. Schwartz et al. (1980a) also found that the levels of oxygenated terpenoids were a greater feeding deterrent than the amount of hydrocarbon terpenoids. Recently, Marko et al. (2008) reported that leaf essential oil concentrations were lowest in *J. communis* heavily browsed by sheep and rabbit, and highest in non-browsed plants.

Juniper foliage intake by goats has been shown to be limited by the presence of monoterpenes (Riddle et al. 1996; Pritz et al. 1997). Monoterpenes have a clearly defined ecological defensive role as feeding deterrents in a variety of mammals and insects (Feeny 1976, Gershenzon et al. 1992). Negative post-ingestive consequences experienced by large ungulates following consumption of high levels of monoterpenes include rumen microbial inhibition (Oh et al. 1967; Schwartz et al. 1980b), hepatic pathogenesis (Estell, et al. 2008, Straka 1993; Bisson et al. 2001; Pritz et al. 1997), and feeding cessation (Dziba et al. 2006; Provenza 1995).

Recently, Adams et al. (2013a) reported on differences between browsed and non-browsed trees of *Juniperus ashei*. In that study, the yields of volatile leaf oil were found to be the major factor associated with browsed and non-browsed samples. The leaf oils of browsed trees were much lower (2.18% DM) than in non-browsed trees (3.47% DM). Associated with oil yields were 12 terpenoids, all of which were at a higher concentration (mg/g) in the non-browsed trees. However, the profile of terpene composition (% total oil) was not very effected, as only 3 terpenoids were significantly different ($P \leq 0.05$). In that study, it appears that selection was based mostly on the total oil yield rather than individual oil components. Of the other variables investigated: extractable condensed tannins (ECT); protein bound

CT (PCT); fiber bound CT; percent total condensed tannin (TCT); percent crude protein (CP); % neutral detergent fiber (NDF); acid detergent fiber (ADF); *in vitro* dry matter digestibility (IVDMD), only PCT and IVDMD showed significant differences between browsed and not-browsed trees.

However, in a related study of browsing on *J. pinchotii*, growing in the same population as *J. ashei* (above), Adams et al. (2013b) found no differences in the yields or composition of the leaf terpenes, tannins, or digestibility characteristics between browsed and un-browsed trees. They concluded that goats were using trees as social gathering points and continued meeting and an occasional bite led to the heavily browsed condition on some trees.

A study of deer browsing (Duncan, Hartley and Iason, 1994) on Sitka spruce (*Picea sitchensis*) found total monoterpene concentration in needles was a negative influence on browsing. Danell, Gref and Yazdani (2008) compared the incidence of moose browsing on Scots pine (*Pinus sylvestris*) with concentrations of leaf mono- and di-terpenes. No correlation was found between mono-terpene concentration and the degree of browsing, but a significant negative correlation was found between the di-terpene, pinifolic acid, and moose browsing.

Winter snow-pack was extremely deep in the foothills around Salt Lake City, UT. Two to three feet of snow prevented deer from browsing on their preferred, shrubs and forbs. As the condition persisted, deer descended into the University of Utah Research Park on Wakara Way, Salt Lake City in search of browse. Several rows of planted Scots pine (*Pinus sylvestris*) from 5 to 11 ft tall were exposed above the snow-pack. Starting with the trees nearest the foothills, the deer began to browse on the lower limbs (up to about 4 ft where they could reach the foliage). However, it soon became obvious that deer were selecting to browse the taller trees. Thus, an opportunity presented itself to examine some phytochemical data from these browsed and lightly browsed trees. The purpose of the present paper is to present data from these phytochemical analyses. Unfortunately, in the intervening years since 1982, the volatile leaf oils have been lost during a lab fire, so no analysis of the oil compositions was possible. However, the phytochemical data is still valid and is examined in this study.

MATERIALS AND METHODS

Plant material - Leaves from six heavily browsed Scots pine (*Pinus sylvestris*) trees and six lightly-browsed trees were sampled on Wakara Way, Salt Lake City, UT.

Essential oils analysis - A portion (100 g FW) of the fresh foliage was kept cool (20°C) and in the dark, then, within 24 hr, steam-distilled for 2 h using a modified circulatory Clevenger-type apparatus (Adams, 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C. Steam distilled leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt. / (oil wt. + oven dried extracted foliage wt.)].

Hexane, methanol extractions - Methods follow that of Adams et al. (1986). Fresh leaves were air dried, (48 h, 70° C) and ground in a Wiley mill to pass a 2 mm mesh screen. A plug of glass wool was placed in an empty Whatman paper thimble (33 mm x 94 mm) and oven dried (48 h, 70° C). To prevent rehydration before pre-weighing, the thimble (containing the glass wool plug) was placed in a desiccator and allowed to cool for 4 hr. Disposable aluminum pans (Kaiser, no. 1016) were used for evaporation of solvents from each extraction, but these were found to contain a volatile coating that would contribute a source of error. Therefore, the aluminum pans were pre-baked at 100° C for 24 h, placed in a desiccator, allowed to cool for 4 h, and then pre-weighed. The air-dried, ground plant material (15-20 g) was placed in the pre-weighed thimble and secured with the corresponding glass wool plug. The plant material in the thimble was extracted sequentially, first with hexane for 20 h in a Soxhlet extractor (followed by oven-drying for 4 h at 100° C to remove traces of hexane from the extracted plant material), and then with

methanol for 20 h in the same extraction apparatus. The extracts were placed in pre-weighed aluminum pans, and the solvents were evaporated in an externally-vented oven. Hexane extracts were concentrated at 100°C for 24 hr before weighing. Methanol extracts were concentrated at 100°C for 48 h and then put in a desiccator and permitted to cool for 4 h before weighing. The extraction thimble, glass wood plug, and marc were oven dried at 100°C for 48 h and placed in a desiccator for 4 h before final weighing for gravimetric determination of yields.

Statistical analyses - Volatile leaf terpenoids (% total oil), % hexane extractables, % methanol extractables, total hexane plus methanol extractables and tree height were compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Principal Component Analysis (PCA) was performed as formulated by the Veldman (1967).

RESULTS AND DISCUSSION

ANOVA revealed (Table 1) a highly significant difference in the yields of volatile leaf terpenoids between heavily browsed trees (0.65%) and for lightly browsed trees (1.06%). No significant differences were found in % hexane extractables or % total hexane + % methanol extractables, but combined hexane + methanol extractables was significantly different (Table 1). As per the field observations, the heavily browsed trees were highly significantly taller (9.08 ft.) compared to the lightly browsed trees (6.00 ft.).

The yield of methanol extractable material was significantly higher in heavily browsed trees (22.73% vs. 20.50%) suggesting that sugars and methanol soluble carbohydrates might be attractive to deer.

Table 1. Comparison of five factors from heavily browsed and lightly browsed trees of *Pinus sylvestris*. Significance, $P \leq 0.05 = *$; $P \leq 0.01 = **$, ns = non significant.

Factor tested	heavily browsed	lightly browsed	F ratio	P, significance
% volatile leaf terpenoids yield (% ODW basis)	0.65	1.06	21.664	0.001, **
% hexane extractables	10.56	10.02	1.001	0.343, ns
% methanol extractables	22.73	20.50	6.704	0.025, *
% total hexane + methanol extractables	33.19	30.68	3.796	0.080, ns
tree height (ft)	9.08	6.00	16.106	0.003, **

The correlation among factors (Table 2) showed a high correlation between % methanol extractables and total extractables which was likely due to the large contribution of methanol to the total extractables. Tree height had a fairly high negative correlation with % oil yield (-0.75). This is not surprising as the smaller trees had higher oil yields (Table 1), suggestive that younger trees produced more oil as a defense.

Table 2. Pearson product correlations among factors in this study.

	% hexane	% methanol	total ext.	tree height	% oil yield
% hexane	1.00	0.54	0.81	0.33	-0.06
% methanol	0.54	1.00	0.92	0.71	-0.43
total ext.	0.81	0.92	1.00	0.59	-0.28
tree height	0.33	0.71	0.59	1.00	-0.75
% oil yield	-0.06	-0.43	-0.28	-0.75	1.00

Factoring the correlation matrix resulted in 3 eigenroots that accounted for 64.7, 24.6 and 7.1% (96.4%) of the variance among the samples. Ordination of the samples revealed eigenroot 1 (correlated with % old yield) is largely responsible for the separation of lightly and heavily browsed trees (Fig. 1).

Finally, it should be noted that after these samples were taken, the deep snow-pack continued and even the smaller, lightly browsed trees became targets of the deer. Browsing continued on all these 12 trees until all the foliage was removed up to about 6 ft into the trees, because the deer stood on their hind legs to reach higher branches. Nearly all the Scots pine trees died in the summer.

So, although a higher yield of leaf terpenoids served as defense, it was not a perfect defense. Because the deer were driven deeper into hunger, they consumed all the leaves available, regardless of oil content or tree height.

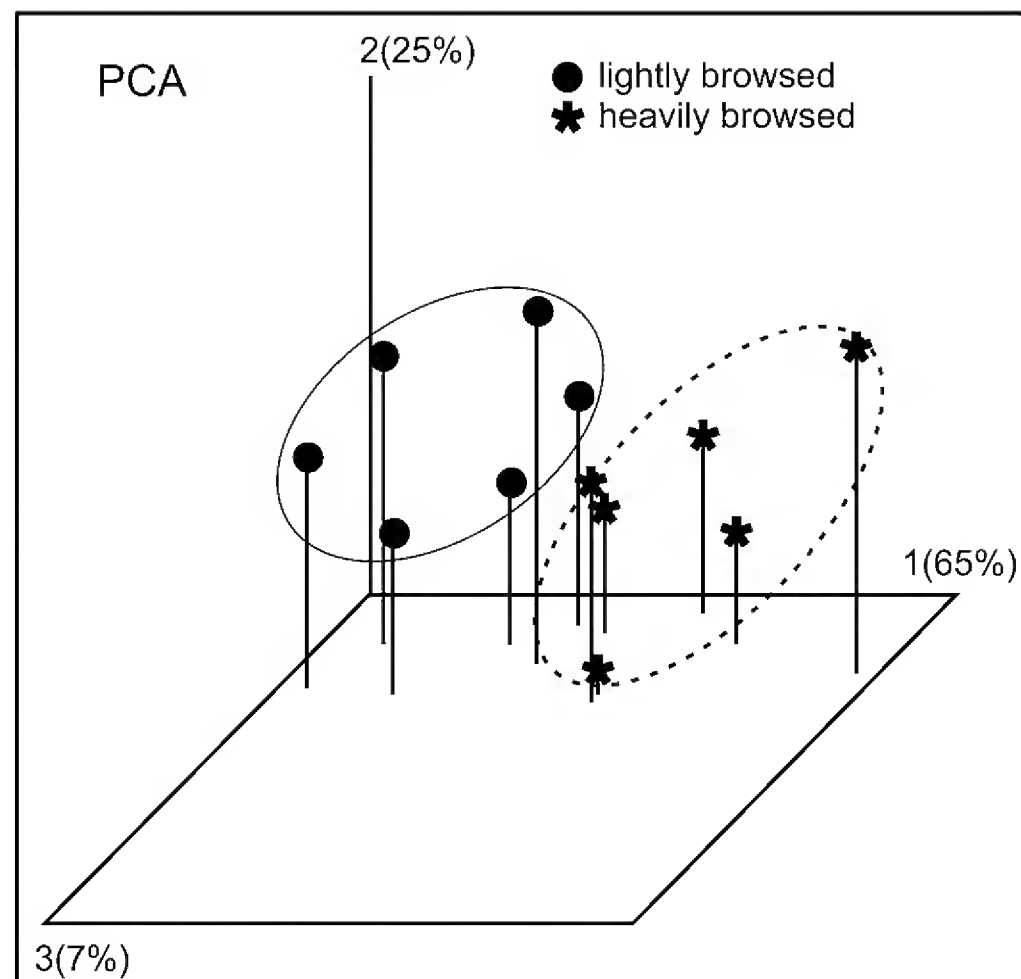


Figure 1. PCA ordination of the heavily and lightly browsed trees.

ACKNOWLEDGEMENTS

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Geographic variation in nrDNA and four cpDNA regions of *Juniperus excelsa* and *J. polycarpus* from Greece, Turkey, Lebanon and Azerbaijan

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ABSTRACT

DNA sequencing of nrDNA, plus four cp DNA regions: petN-psbM, trnS-tenG, trnD-trnT and trnL-trnF of *J. excelsa* and *J. polycarpus* from Azerbaijan, Greece, Lebanon and Turkey revealed that putative *J. excelsa* from Azerbaijan is *J. polycarpus* (= *J. excelsa* subsp. *polycarpus*). Two Lebanon *Juniperus* populations from Afqa (1306 m) and Wadi El Njass (2287 m), previously shown to be divergent in their microsatellites, were found to be *J. excelsa* and *J. polycarpus*, respectively. This is the first report of the occurrence of *J. polycarpus* in Lebanon. To aid comparisons, DNA sequences from *J. seravschanica* and *J. polycarpus* var. *turcomanica* and *J. procera* were included in the Bayesian analysis. Published on-line www.phytologia.org *Phytologia* 96(2): 89-95 (April 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus excelsa*, *J. polycarpus* var. *polycarpus*, *J. polycarpus* var. *turcomanica*, *J. seravschanica*, DNA sequencing, nrDNA, petN-psbM, trnS-tenG, trnD-trnT, trnL-trnF.

Recently, Douaihy et al. (2011), using 3 microsatellites of putative *J. excelsa*, found the Nei genetic distance separated their 12 populations into 3 groups: Lebanon (Leb 1, 2, 4, 5), Lebanon (Leb 3, 6) and the Crimea, Cyprus, Greece and Turkey populations (Fig. 1). PCO of the data removed 38.8% and 27.5% on the first two axes. Ordination clearly shows: Lebanon (Leb 1, 2, 4, 5), Lebanon (Leb 3, 6) and the Crimea, Cyprus, Greece and Turkey populations (Fig. 2). El Njass (Leb 3, 2287 m) and Aarsal (Leb 6, 2180 m) are from higher elevations in Lebanon. Examination of specimens (RPA) from Afqa, 1300 m and El Njass, 2287 m, found the leaves of Afqa plants had very fine, small leaves and were bluish green similar to *J. excelsa* from Greece. The leaves of the El Njass plants were larger, coarse and yellowish green similar to *J. polycarpus* from Armenia and *J. p.* var. *turcomanica* from Turkmenistan.

In a comprehensive study of the reproductive ecology of *Juniperus* in Lebanon, Douaihy et al. (2013) reported differences between the higher (El Njass, Aarsal) and lower (Afqa, etc.) populations in cones density classes, frequencies of adult and juvenile trees, and dioecious (El Njass, Aarsal) vs. monoecious (Afqa, etc.) individuals. Interestingly, Adams (2014) describes *J. excelsa* as monoecious or dioecious and *J. polycarpus* as dioecious.

Juniperus excelsa M.-Bieb. grows from Greece to Turkey and perhaps as far east as Azerbaijan (Fig. 1). Farjon (2005, 2010) treated *J. polycarpus*, *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpus*. However, Adams and Schwarzbach (2012) and Adams (2013), utilizing

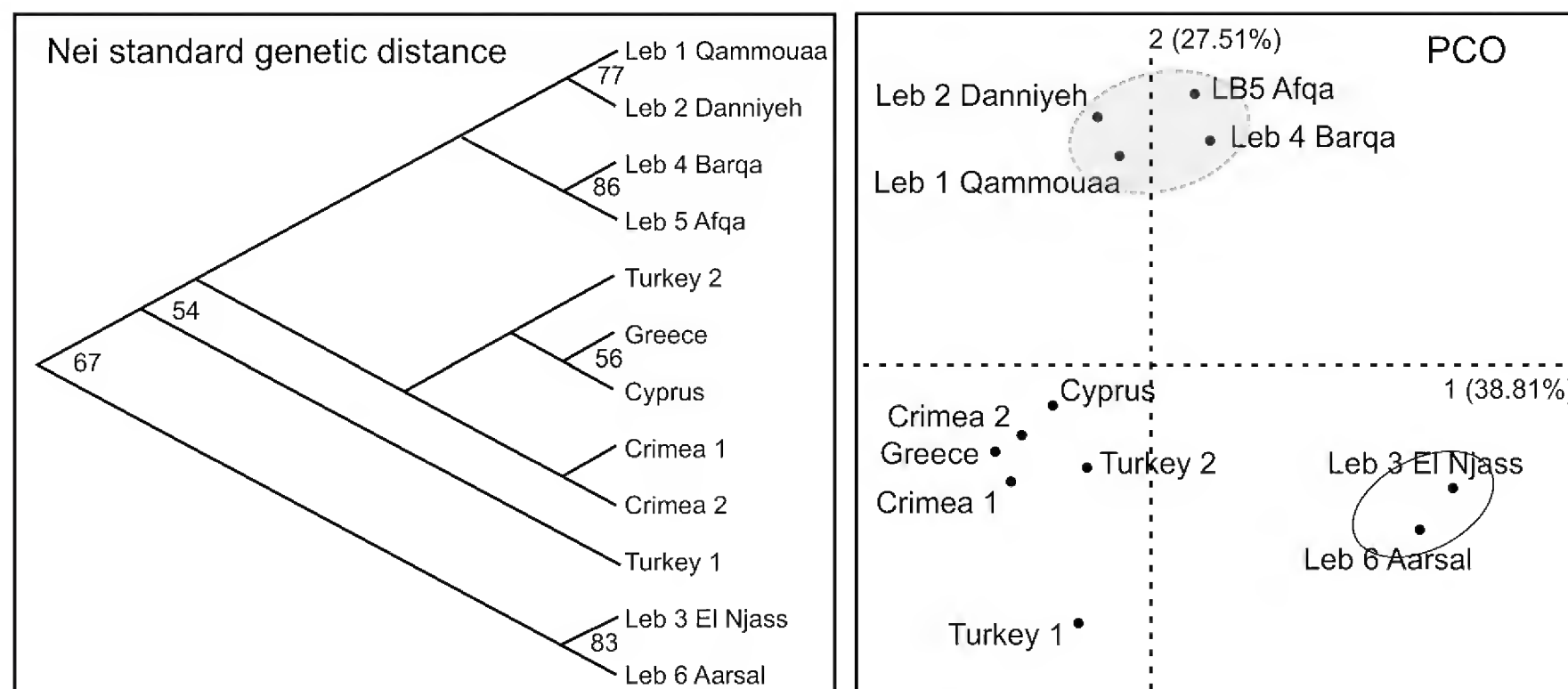


Figure 1. Nei distance diagram. Note the Lebanon (Leb 1, 2, 4, 5) at top and Leb 3, 6 at bottom.

Figure 2. PCO, microsatellite data. *J. excelsa* is ordinated into 3 groups.

DNA sequence data, recognized *J. excelsa* in addition to *J. polycarpus*, *J. p.* var. *turcomanica* and *J. seravschanica*. Adams and Hojjati (2012) and Adams, Hojjati and Schwarzbach (2014), using sequences from 4 gene regions, failed to verify the occurrence of *J. excelsa* in Iran, but did find *J. polycarpus*, *J. p.* var. *turcomanica* and *J. seravschanica* in Iran. Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had none or only one SNP difference compared with *J. polycarpus* var. *polycarpus* from Armenia and was concluded to be *J. polycarpus* (Adams and Hojjati, 2012).

The distributions of *J. excelsa* and *J. polycarpus* (stricto sensu) are shown in Figure 3. It is difficult to distinguish these taxa and they have been treated as conspecific (Farjon, 2005, 2010; Douaihy et al, 2011). The distribution of *J. excelsa* into Armenia, Azerbaijan and Iran has proved difficult to determine by modern methods of DNA sequencing and leaf essential oil data, due to the lack of access to these regions. Recently, materials were obtained of *J. excelsa*/ *J. polycarpus* from Lebanon and *J. excelsa*/ *J. polycarpus* from Azerbaijan. This afforded the opportunity to further examine geographic variation in the DNA sequences of both *J. excelsa* and *J. polycarpus*.

The purpose of the paper is to examine nrDNA, and 4 cp DNA regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF and report on variation in *J. excelsa* and *J. polycarpus*.

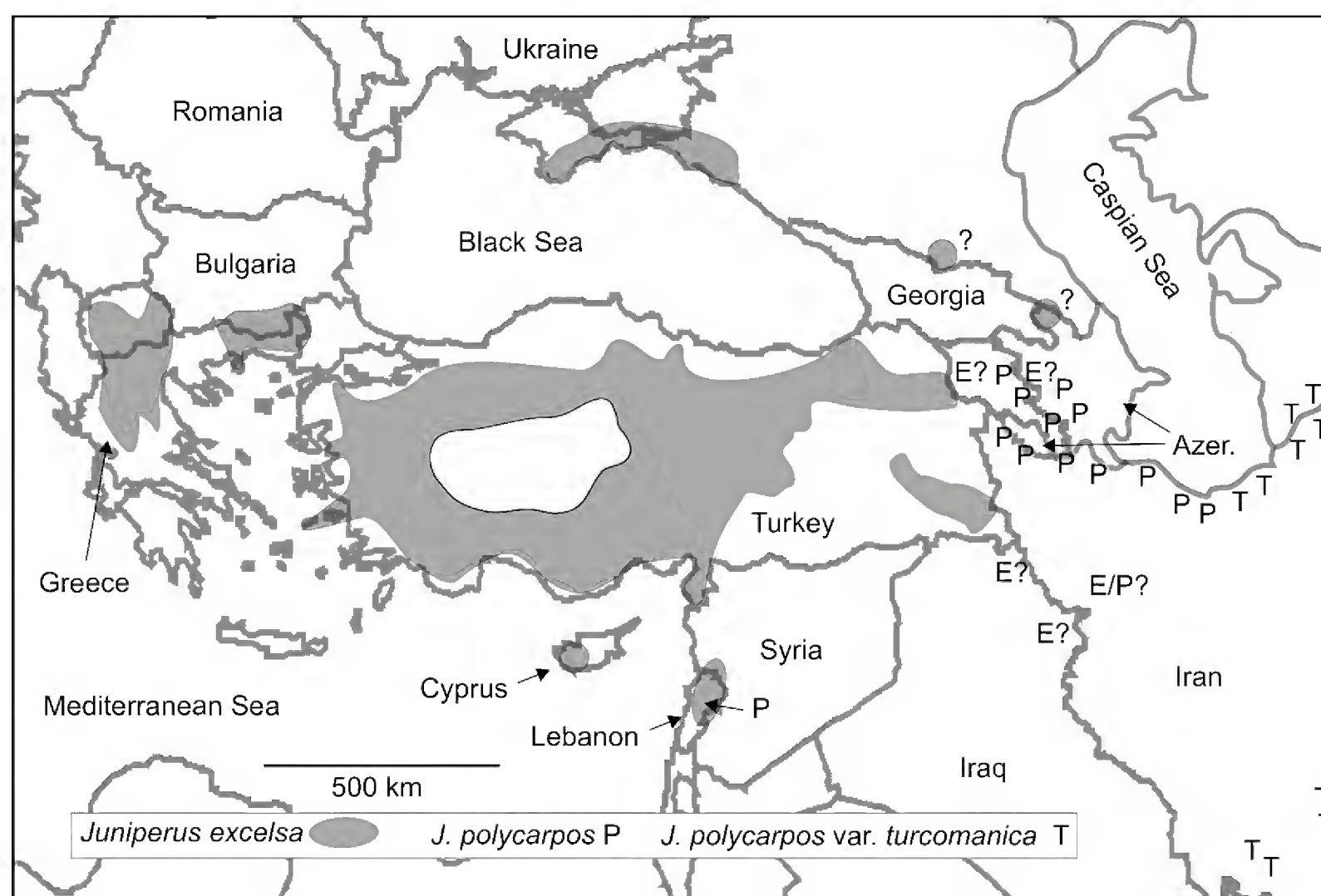


Figure 3. Distribution of *J. excelsa*, *J. polycarpus* var. *polycarpus* (P) and *J. p.* var. *turcomanica* (T) . Questionable locations of *J. excelsa* and *J. polycarpus* are indicated by E? and P? (modified from Adams, 2014).

MATERIALS AND METHODS

Plant material -

J. excelsa: Eskisehir, Turkey, 820m, Adams 13193 (9433-9435), Bulgaria, 356 m, Adams 14056 (13720-13724), Alex Tashev, 2012-1-JE -5-JE, 42° 01' 22.0" N; 24° 28' 03.1" E, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune"; Lemos, Greece, 1100m, Adams 6031 (5983-5985, 5987), Cyprus, Adams 13487, A. K. Christou s.n., bulk 5 trees; Afqa, Lebanon, 1306 m, Adams 14155-14157, Bouchra Douaihy 1-3, 34° 04' 58.12"N, 35° 53' 08.52"E, 4 Nov 2013,

J. polycarpus: Armenia, Lake Sevan, 1900m, Adams 13194 (8761-8763); Azerbaijan, 177-231m, Adams 14162-14171, Vahid Farzaliyev 1-10, 40° 44' 41.05" N; 47° 35' 19.14" E, Dec 2013; Lebanon, Wadi El Njass, 2287m, Adams 14158-14161, Bouchra Douaihy 4-7, 34° 20' 47.79"N, 36° 05' 45.54"E, 14 Nov 2013.

J. polycarpus var. *turcomanica*: Adams 13197 (8758-90), Kopet Mtns., Turkmenistan;

J. procera: Adams 6184-6188, Guder, Ethiopia;

J. seravschanica: Adams 13195 (8483-85), Pakistan.

Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams,

Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp regions petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF yielded 4430 bp of data. The Bayesian consensus tree (Fig. 4) shows *J. seravschanica*, *J. polycarpus*, *J. p. var. turcomanica*, *J. procera* and *J. excelsa* in well-supported clades. All of the samples from Azerbaijan are closely nested with *J. polycarpus*, Armenia along with the El Njass, Lebanon (Adams 14161) sample (Fig. 4). Three other El Njass samples (14158, 58, 60) appear to be intermediate between *J. polycarpus* and *J. p. var. turcomanica* (Fig. 4). The three Afqa, Lebanon samples (14155, 56, 57) are in the clade with *J. excelsa* from Greece and Turkey (Fig. 2).

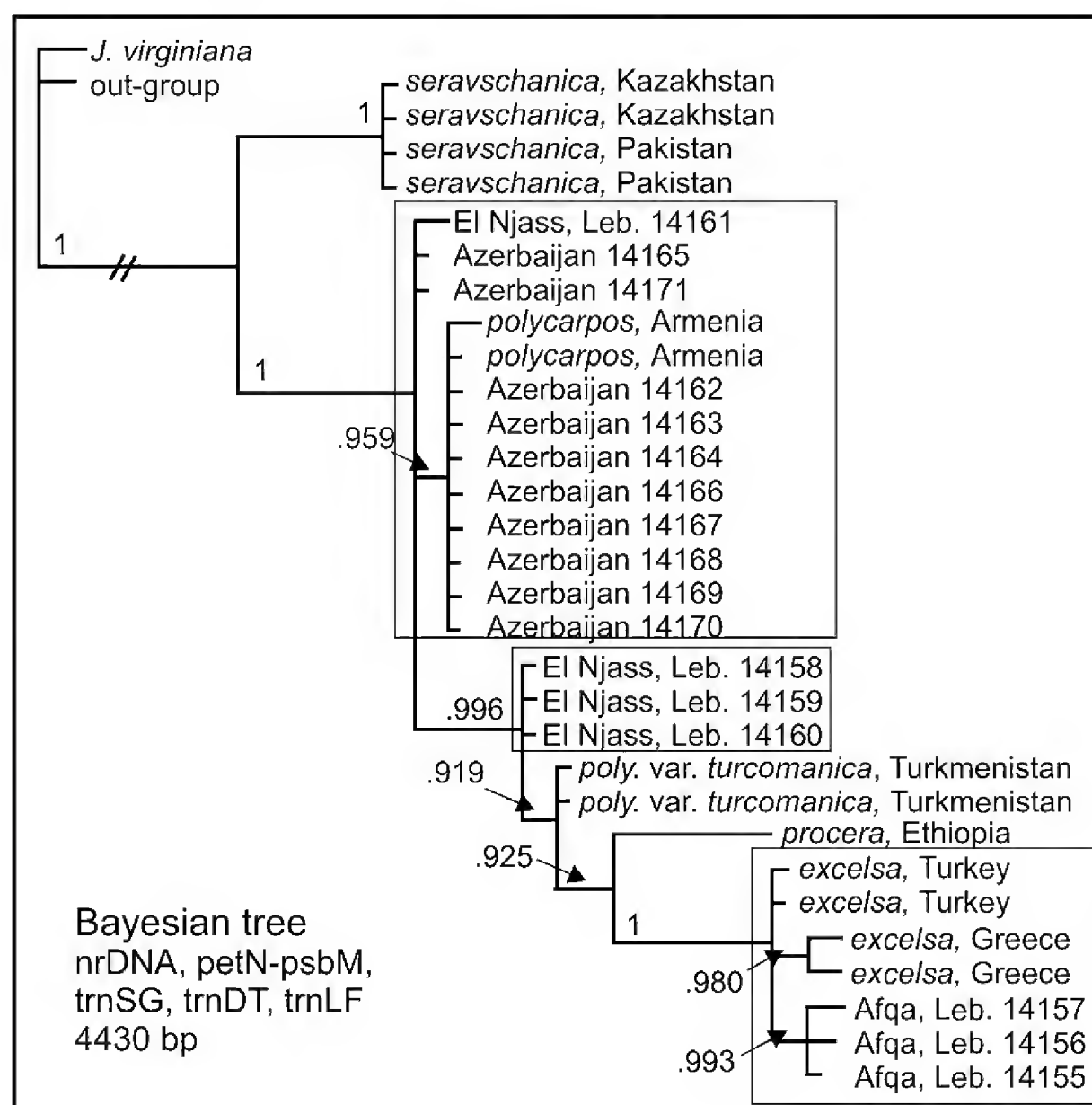


Figure 4. Bayesian tree based on nrDNA (ITS) and four cp regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF (4430 bp). Numbers at the branch points are posterior probabilities.

To examine the magnitude of the differences among the taxa, aligned sequences were examined for single nucleotide polymorphisms plus indels (= mutation events, MEs). Analysis revealed a total of 114 MEs, with 18 occurring only once and 96 occurring 2 or more times (i.e., multiple). Although the single MEs may be of interest in examining mutation rates, only the 96 multiple occurrence MEs were utilized in the construction of a minimum spanning network (Fig. 5). All the samples from Azerbaijan and El Njass, Lebanon are found between *J. polycarpus*, Armenia and *J. p. var. turcomanica*, Turkmenistan (Fig. 5). Three of the El Njass trees differ by only 1 ME from *J. p. var. turcomanica*. The fourth El Njass sample (14161) differs by only 1 ME from Azerbaijan samples (Fig. 5). Two Azerbaijan trees (14165, 14171) differ by 1 ME from the other 8 Azerbaijan samples (Fig. 5). Clearly the El Njass and Azerbaijan samples are *J. polycarpus*.

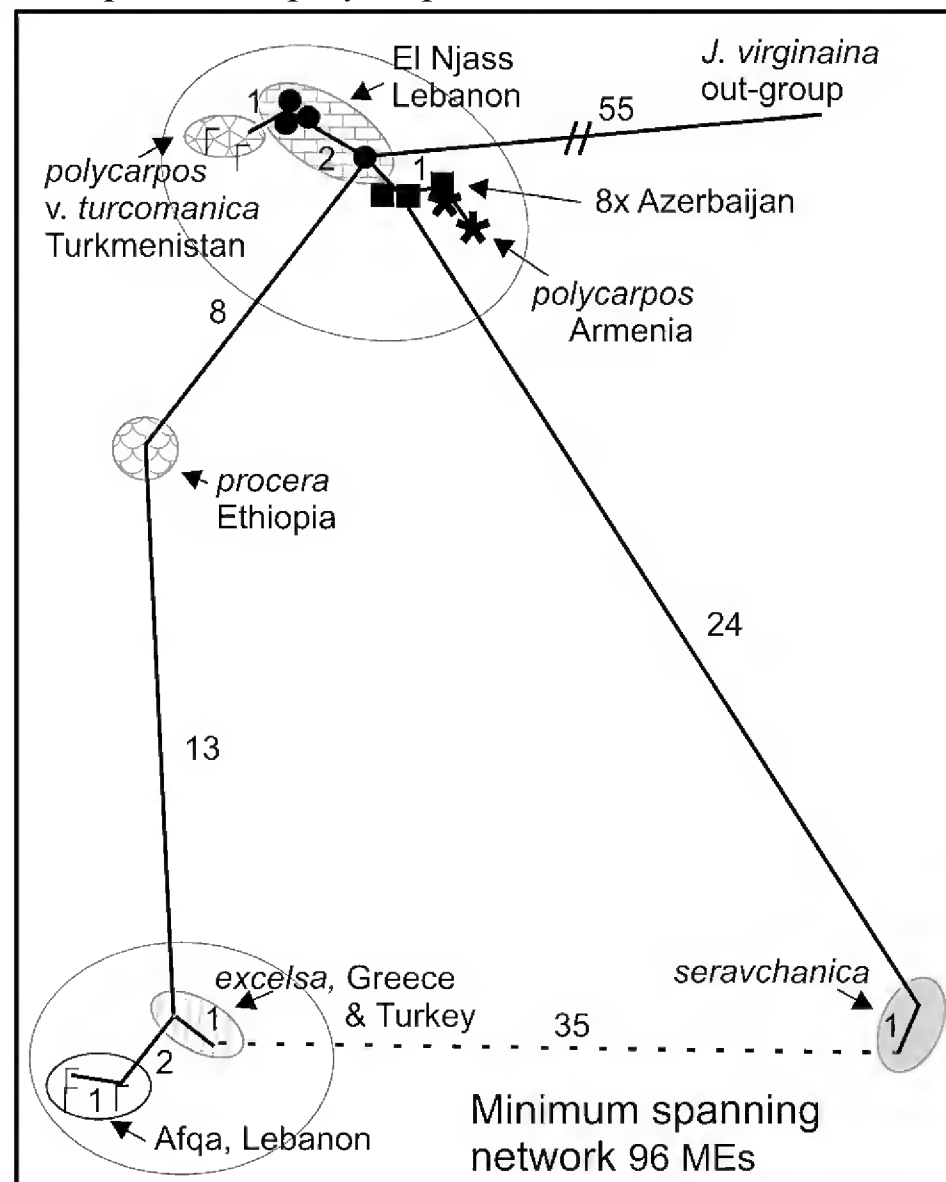


Figure 5. Minimum spanning network based on 96 MEs. Numbers next to links are the number of MEs separating the nodes. The dashed line is the shortest link between *J. excelsa* and *J. seravschanica* (35 MEs).

Juniperus procera differs by 8 MEs from *J. polycarpus* and 13 MEs from *J. excelsa* (Fig. 5), whereas *J. seravschanica* differs by 24 MEs from *J. polycarpus*. The three trees from Afqa, Lebanon differ by only 2 MEs from *J. excelsa*, Turkey (Fig. 5).

Overlaying a minimum spanning network onto a distribution map gives one a perspective of the geographic trends (Fig. 6). The Afqa, Lebanon *J. excelsa* population differs by 2 MEs from Eskisehir, Turkey, which in turn, differs by only 1 MEs from the Lemos, Greece population (Fig. 6). The other Lebanon populations that group with Afqa (Figs. 1, 2) are probably *J. excelsa*.

However, the Wadi El Njass, Lebanon (2287 m) population, although very near Afqa, is *J. polycarpus* and differs by 1 to 3 MEs from *J. p. var. turcomanica*, Turkmenistan and by 1 to 2 MEs from *J. polycarpus*, Armenia (Fig. 6). The *J. excelsa*, Afqa population is only about 100 - 150 km from other

J. excelsa populations (Fig. 6), but the Wadi El Njass, *J. polycarpus* population is 700 to 1000 km from the nearest *J. polycarpus* population. Yet, it differs by only 1 to 3 MEs.

The discovery of the *J. polycarpus*, Wadi El Njass population in Lebanon was unexpected. The trees at nearby Aarsal are probably *J. polycarpus* (Figs. 1, 2). Lebanon may have been a refugium for *J. polycarpus* during the Pleistocene ice age. This discovery also points to the need for more extensive field work on the isolated mountains of northern Syria, Iraq and Iran to determine if other disjunct populations of *J. polycarpus* (or *J. excelsa*) exist.

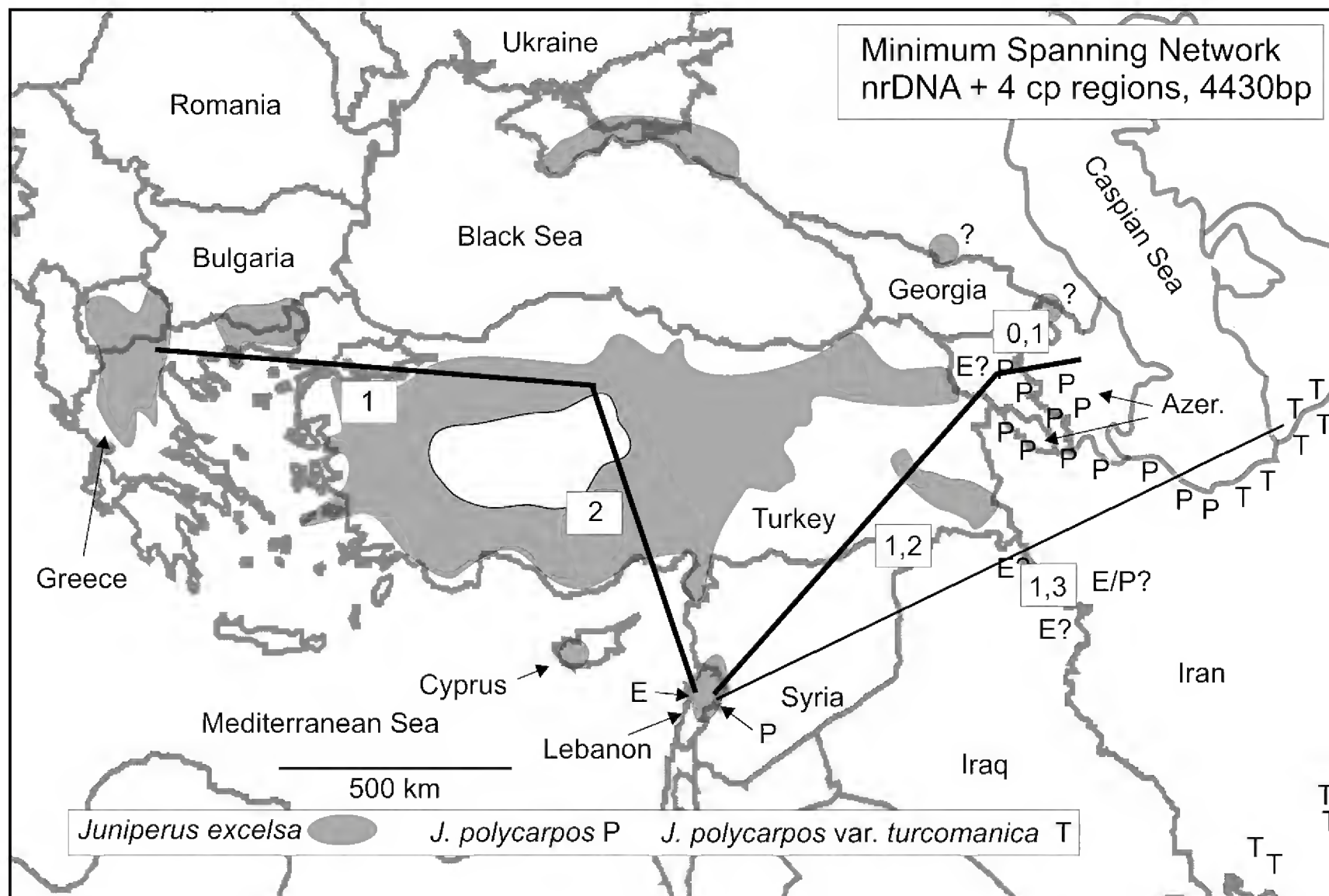


Figure 6. Minimum spanning network mapped onto the distributions of *J. excelsa* and *J. polycarpus*. Numbers next to lines are the number of MEs.

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Geographic variation in the volatile leaf oils of *Juniperus excelsa* and *J. polycarpus***Robert P. Adams**

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ABSTRACT

Analyses of *J. excelsa* revealed the volatile leaf oils from Bulgaria and Greece were higher in α -pinene, limonene and β -phellandrene than populations from Turkey, Cyprus and Lebanon. Otherwise, there was little variation in the oils between these populations. Cedrol was a major component in each of the populations, ranging from 22.6 to 29.3%. Analyses of *J. polycarpus* var. *polycarpus* from Azerbaijan revealed the presence of high cedrol and zero cedrol chemotypes. The high cedrol chemotype was similar to the oil from Armenia. The Azerbaijan zero cedrol chemotype was similar to the oil from El Njass, Lebanon. The compositions of oils of *J. polycarpus* var. *turcomanica* and *J. seravschanica* were compared with the oils of *J. excelsa* and *J. polycarpus* var. *polycarpus*. Published on-line: www.phytologia.org *Phytologia* 96(2): 96-106 (April 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus excelsa*, *J. polycarpus* var. *polycarpus*, *J. polycarpus* var. *turcomanica*, *J. seravschanica*, geographic variation, leaf oils, terpenes, cedrol, α -pinene, limonene.

Adams et al. (2013) recently examined geographic variation in *J. excelsa* and reviewed the leaf essential oil literature. They found *J. excelsa* oils from Bulgaria and Greece to have lower α -pinene (24.3, 21.6%) than oils from Turkey and Cyprus (41.7, 41.8%) plus many other quantitative differences. Cedrol was present at 25.4 to 29.3% in each population and ranged from 11.3% to 35.8% in all 12 trees examined.

The oils of *J. polycarpus* var. *polycarpus*, *J. p.* var. *turcomanica* and *J. p.* var. *seravschanica* (now *J. seravschanica*) have been the subject of several studies by Adams et al. (2008). Initial reports on *J. polycarpus* (Adams 2001; Adams et al. 2008) oils from Armenia (Lake Sevan) were high in α -pinene and cedrol. Recently, Adams and Hojjati (2013) analyzed the leaf essential oils of *J. polycarpus* var. *turcomanica* and *J. seravschanica* from central and southern Iran and included analyses of *J. polycarpus*, (Armenia) and *J. excelsa* (Eskisehir, Turkey).

The distributions of *J. excelsa* and *J. polycarpus* (stricto sensu) are shown in Figure 1. It is difficult to distinguish these taxa and they have been treated as conspecific (Farjon, 2005, 2010; Douaihy et al, 2011). Douaihy et al. (Fig. 4, 2011), using 3 microsatellites, found the *J. excelsa* ordinated into 3

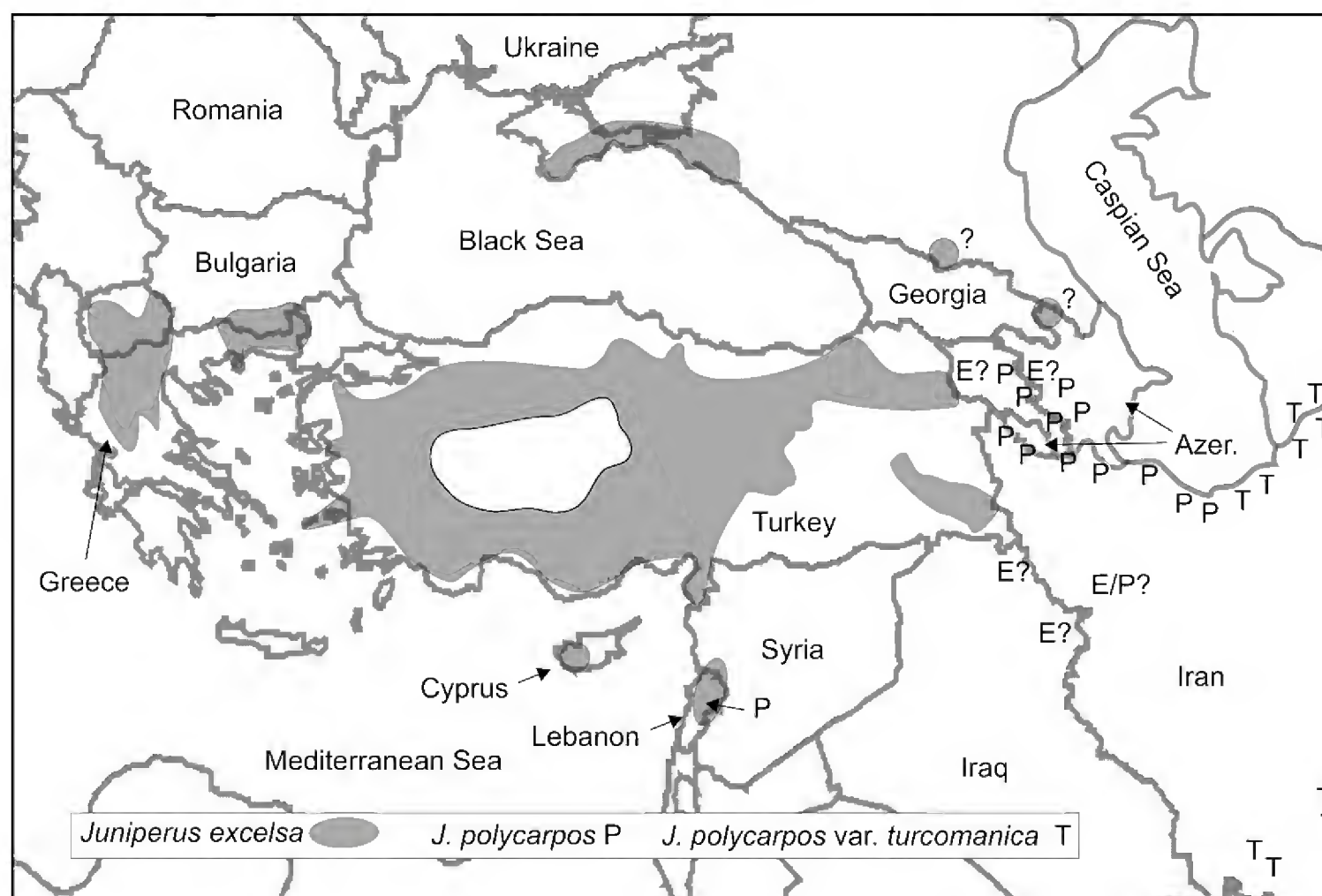


Figure 1. Distribution of *J. excelsa*, *J. polycarpus* var. *polycarpus* and *J. p.* var. *turcomanica* (modified from Adams (2014)). Questionable populations of *J. excelsa* and *J. polycarpus* are indicated by E? and P?.

groups: Greece, Ukraine (Crimea), Turkey, Crete; Lebanon (moderate elevations: Qammouaa, Danniyeh, Barqa, Afqa) and Lebanon (two very high elevations, Aarsal 2180 m; El Njass 2287 m). Recently, Adams et al. (2014), using 4430 bp of DNA sequence data, have shown that the trees at Afqa, 1307 m, are *J. excelsa* and the trees at El Njass, 2287 m, are *J. polycarpus*, but appearing intermediate to *J. p.* var. *turcomanica*.

Recently, leaf materials suitable for distillation were obtained of *J. excelsa* and *J. polycarpus* from Lebanon and *J. polycarpus* from Azerbaijan. This afforded the opportunity to further examine geographic variation in the volatile leaf oils of both *J. excelsa* and *J. polycarpus*.

MATERIALS AND METHODS

Plant material -

J. excelsa: Eskisehir, Turkey, 820m, Adams 13193 (9433-9435), Bulgaria, 356 m, Adams 14056 (13720-13724), Alex Tashev, 2012-1-JE -5-JE, 42° 01' 22.0" N; 24° 28' 03.1" E, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune"; Lemos, Greece, 1100m, Adams 6031 (5983-5985,

5987), Cyprus, Adams 13487, A. K. Christou s.n., bulk 5 trees; Afqa, Lebanon, 1306 m, Adams 14155-14157, Bouchra Douaihy 1-3, 34° 04' 58.12"N, 35° 53' 08.52"E, 4 Nov 2013, *J. polycarpus*: Armenia, Lake Sevan, 1900 m, Adams 13194 (8761-8763); Azerbaijan, 177-231m, Adams 14162-14171, Vahid Farzaliyev 1-10, 40° 74' 47.6" N; 40° 58' 55" E, Dec 2013; Lebanon, Danniye-Wadi El Njass, 2287 m, Adams 14158-14161, Bouchra Douaihy 4-7, 34° 20' 47.79"N, 36° 05' 45.54"E, 14 Nov 2013.
J. polycarpus var. *turcomanica*: Adams 13197 (8758-90), Kopet Mtns., Turkmenistan;
J. seravschanica: Adams 13195 (8483-85), Pakistan.
 Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The compositions of the leaf oils for the five populations of *J. excelsa* are given in Table 1. The major components of the oils are cedrol (22.6 - 29.3%), α -pinene (21.6 - 41.8%, limonene (0.5 - 13.3%), and β -phellandrene (0.4 - 9.2%). The oils from Bulgaria and Greece are higher in α -pinene, limonene and β -phellandrene than populations from Turkey, Cyprus and Lebanon. Otherwise, there was little variation in the oils between these populations of *J. excelsa*. Variation in cedrol among *J. excelsa* trees, ranges from 11.3% (Eshisehir, Table 2) to 35.8% (Greece, Table 2). In contrast to *J. polycarpus* (see below) where all trees contained considerable amounts of cedrol (and associated cedarwood oil components).

The leaf oil of *J. polycarpus* (El Njass, Lebanon) is included in Table 1 and its oil differs from *J. excelsa* by lacking the typical cedarwood compounds α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc. (Adams, 2014). In addition, *J. polycarpus* has a larger concentration of α -pinene (55.6%), plus (4Z)-decen-1-ol, (E)-caryphyllene, α -muurolene, γ -cadinene, elemol, germacrene B, germacrene D-4-ol, junenol, epi- α -cadinol, epi- α -muurolol, α -murrolol, α - and β -eudesmols and shyobunol.

Analyses of *J. polycarpus* var. *polycarpus* from Azerbaijan revealed the presence of high cedrol and low (trace amounts) cedrol (and cedarwood components) chemotypes. Most *Juniperus* species produce two kinds of essential oils: leaf oils and heartwood oils and these oils have few components in common (Adams, 1991). *Juniperus excelsa*, along with *J. foetidissima*, *J. polycarpus*, and *J. seravschanica* have leaf oils that contain significant amounts of the heartwood oil components (cf. α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc., see Adams, 2014). For example, Adams (1990) reported 4.4, 0.2, trace and 8.3% cedrol in the leaf oils from four trees of *J. foetidissima* from Greece. Whereas, Tunalier et al. (2004) reported 13.0 and 12.2% of cedrol and widdrol in the stem heartwood of *J. foetidissima* from Turkey. Ucar and Balaban (2002) analyzed the sapwood (white wood) of *J. excelsa*, Turkey, and reported the oil to contain 22.5% widdrol and 9.0% cedrol (these components are difficult to separate on non-polar columns and the mass spectra are nearly identical, so their identification is often problematic).

When *Juniperus* species contain heartwood components in the leaf oils, it is common to find chemical polymorphisms in cedrol (and associated heartwood terpenes) between trees. That is the case for *J. polycarpos* from Azerbaijan with cedrol in five trees ranging from 24.8 to 34.4% (hi cedrol chemotype, Table 3) and the cedrol of five other trees ranging from 0.00 to 0.03% (low cedrol chemotype, Table 3). The samples from Armenia ranged from 26.0 to 34.3% in cedrol. In the trees from El Njass, Lebanon, cedrol ranged from 0.00 to 0.01%. Trace amounts of cedrol may be due to free radical interactions rather than enzyme based reactions (or even cross-contamination during distillation, and GC analysis). It is interesting that in trees of *J. p. var. turcomanica* from Turkmenistan, only trace amounts of cedrol were found (Table 4), but in a population from Fasa, Iran, two chemotypes were found (0.00 to 0.1%, and 10.1, 13.8%, Table 4). Adams and Hojjati (2013) found *Juniperus* in the Fasa area to be quite variable and it seems likely that some of the samples are hybrids or introgressants with *J. p. var. polycarpos* in that population (see Adams, Hojjati and Schwarzbach, 2014 for DNA analyses).

Geographic variation in the leaf terpenoids of *J. polycarpos* is difficult to assess due to the high and low cedrol chemotypes in populations. The compositions of the leaf oils for the four populations of *J. polycarpos* are given in Table 4. The Azerbaijan high cedrol chemotype oil was similar to the high cedrol oil from Armenia (Table 4). The Azerbaijan low cedrol chemotype was similar in composition to the low cedrol oil from El Njass, Lebanon. The higher concentrations of several major compounds in El Njass and Azerbaijan, low cedrol) oils seems to be explained by the presence of cedrol (and other cedarwood compounds) in the high cedrol oils of Azerbaijan and Armenia. For example, removing the cedrol and other cedarwood components from Azerbaijan and Armenia, high cedrol oils, shows the revised concentrations of α -pinene, germacrene B and shyobunol to be quite similar (Table 5) to that found in El Njass and Azerbaijan (low cedrol). However, the correction for cedarwood oil components was not sufficient to produce uniform levels for δ -cadinene, germacre-4-ol, and α -eudesmol (Table 5). It appears the terpene synthase genes for production of cedrol (and related compounds) interferes with the production of some other compounds (cf. δ -cadinene, germacre-4-ol, and α -eudesmol) between Azer lo ced and Azer hi ced, Table 5).

The low-cedrol leaf oils of *J. polycarpos* var. *polycarpos* (Njass, Azerbaijan) and *J. p. var. turcomanica* are nearly identical in compositions. (Table 4). DNA sequence data (Adams et al., 2014) showed the El Njass trees to be somewhat intermediate between *J. polycarpos* var. *polycarpos* and *J. p. var. turcomanica*. This is shown in α -pinene as it is intermediate in concentration. The oil of El Njass does show complementary amounts for some terpenes (var. *turcomanica*, El Njass, var. *polycarpos*): δ -3-carene (t, 2.1, 2.3); terpinolene (0.8, 0.8, 1.2); trans-verbenol (0, 0.4, 0.8); 4Z-decen-1-ol (0, 0.2, 0.2); β -elemene (0.2, 0.2, 0); epi-cubebol (0.5, 0.5, 0); germacrene D-4-ol (8.9, 4.0, 3.7); junenol (0, 0.5, 0.2); α -cadinol (3.6, 1.1, 0.8); manoyl oxide (0, 0.6, 0.2) and 4-epi-abietal (1.9, 0.7, 0.9). Additional research on the relationship of the El Njass trees to *J. polycarpos* var. *polycarpos* and *J. p. var. turcomanica* should prove interesting.

In spite of the presence or absence of cedarwood components in the leaf oils in some populations of *J. polycarpos* var. *polycarpos*, there appear to be only minor geographical differences in the leaf oils. The leaf oil of *J. p. var. turcomanica* is most similar to the low cedrol oils found in El Njass and Azerbaijan (Table 4).

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Table 1. Leaf essential oils from populations of *J. excelsa*. Afqa, Lebanon, *Adams 14155-14157*; Bulgaria: *Adams 13720-13724*; Lemos, Greece: *Adams 5983-5985, 5987*, Eskisehir, Turkey; *Adams 9433-9435* and Cyprus, *Adams 13487* (bulk, 5 trees) and *J. polycarpus*, El Njass, Lebanon, *Adams 14158-14161*. Components that are typical of heartwood oil are highlighted in italics.

KI	Compound	<i>J. excelsa</i>					<i>polycarpus</i>
		Kritchim Bulgaria	Lemos Greece	Eskisehir Turkey	Cyprus	Afqa, Lebanon	El Njass Lebanon
921	tricyclene	t	0.1	0.2	0.1	0.1	0.1
932	<i>α-pinene</i>	24.3	21.6	41.7	41.8	37.9	55.6
945	<i>α</i> -fenchene	0.1	0.1	0.3	t	0.5	0.2
946	camphene	0.2	0.3	0.2	0.1	0.2	0.3
953	thuja-2,4(10)-diene	t	t	0.1	t	t	0.1
961	verbenene	t	t	t	t	-	-
969	sabinene	t	0.2	0.1	0.1	t	t
974	<i>β</i> -pinene	0.5	0.5	0.7	0.4	0.6	0.8
988	myrcene	1.4	1.2	1.2	0.5	1.4	1.5
1002	<i>α</i> -phellandrene	t	t	0.1	t	t	t
1008	<i>δ</i> -3-carene	0.8	1.7	5.3	t	10.8	2.1
1014	<i>α</i> -terpinene	t	t	0.1	t	0.1	t
1020	<i>p</i> -cymene	0.5	0.4	0.6	0.1	0.2	0.2
1024	sylvestrene	-	-	-	-	t	-
1024	<i>limonene</i>	11.3	13.2	1.2	0.5	0.8	1.0
1025	<i>β</i>-phellandrene	7.5	9.2	0.9	0.4	0.8	0.7
1044	(E)- <i>β</i> -ocimene	t	t	t	t	t	t
1054	<i>γ</i> -terpinene	0.5	0.4	0.5	0.3	0.4	0.3
1065	cis-sabinene hydrate	t	t	t	t	-	-
1086	terpinolene	0.8	0.7	1.1	0.3	1.9	0.8
1095	linalool	-	-	-	-	t	0.4
1097	trans-sabinene hydrate	t	t	t	t	-	-
1112	3-me-3-butenyl-methyl butanoate	0.4	0.1	t	t	0.1	0.1
1114	endo-fenchol	0.2	0.2	t	t	t	-
1118	cis-p-menth-2-en-1-ol	-	-	0.1	t	-	-
1122	<i>α</i> -campholenal	0.2	0.1	0.5	0.1	0.2	0.2
1135	trans-pinocarveol	0.4	0.2	0.8	0.1	0.2	0.2
1137	trans-verbenol	-	-	0.2	-	-	0.3
1141	camphor	0.7	0.5	1.2	0.2	0.8	0.4
1145	camphene hydrate	t	t	0.1	t	t	0.1
1158	trans-pinocamphone	-	-	-	-	0.1	-
1165	borneol	-	-	-	-	-	0.2
1166	<i>p</i> -mentha-1,5-dien-8-ol	t	t	-	t	t	-
1172	cis-pinocamphone	t	t	0.2	t	t	-
1174	terpinen-4-ol	t	0.2	0.1	0.1	-	t
1178	naphthalene	t	t	0.1	t	-	-
1179	<i>p</i> -cymen-8-ol	t	t	0.1	t	-	-
1186	<i>α</i> -terpineol	t	t	t	t	-	t
1193	(4Z)decenal	-	-	-	-	-	0.2
1195	myrtenol	-	-	-	-	-	0.1
1204	verbenone	t	t	0.2	t	0.1	0.2
1215	trans-carveol	0.2	0.1	0.2	t	0.1	0.1
1218	endo-fenchyl acetate	t	0.2	0.1	t	-	-
1241	hexyl isovalerate	-	-	-	-	-	0.1

KI	Compound	<i>J. excelsa</i>					<i>polycarpus</i>
		Kritchim Bulgaria	Lemos Greece	Eskisehir Turkey	Cyprus	Afqa, Lebanon	El Njass Lebanon
1249	piperitone	t	t	0.1	t	0.1	-
1255	(4Z)-decen-1-ol	-	-	-	-	-	0.2
1260	3-me-3-butenol hexanoate	0.2	0.1	-	-	t	-
1274	pregeijerene B	-	-	-	-	-	-
1284	bornyl acetate	t	0.6	0.4	0.1	0.1	0.3
1292	(2E,4Z)-decadienal	t	0.3	0.1	t	-	-
1319	(2E,4E)-decadienol	3.9	3.6	2.4	2.3	3.5	-
1335	δ -elemene	-	-	-	-	-	0.2
1374	α -copaene	-	-	-	-	-	0.1
1387	β -bourbonene	t	0.1	0.1	t	t	0.1
1389	β -elemene	-	-	-	-	-	0.2
1390	7-epi-sesquithujene	0.2	0.1	0.1	0.3	-	-
1410	α -cedrene	1.0	1.1	0.8	1.7	1.2	-
1413	β -funebrene	1.0	0.9	0.7	1.8	0.6	-
1417	(E)-caryophyllene	-	-	-	-	-	0.7
1419	β -cedrene	1.1	1.0	0.5	1.0	0.7	-
1429	cis-thujopsene	0.3	0.4	0.3	0.8	0.3	-
1434	γ -elemene	-	-	-	-	-	0.2
1449	trans-sibirene	-	-	-	-	-	0.2
1451	trans-muurola-3,5-diene	0.2	0.1	0.1	0.7	t	-
1452	α -humulene	0.1	0.2	0.1	0.2	t	0.1
1454	(E)- β -farnesene	0.1	0.2	0.2	0.3	0.1	-
1469	β -acoradiene	0.1	0.1	0.2	0.4	0.1	-
1475	trans-cadina-1(6),4-diene	0.4	0.3	0.2	0.7	0.1	t
1478	γ -muurolene	-	-	-	-	-	0.2
1480	germacrene D	0.8	0.8	0.6	1.2	1.2	1.1
1489	β -selinene	-	-	-	-	-	0.2
1493	trans-muurola-4(14),5-diene	0.6	0.4	0.2	1.5	0.1	-
1493	epi-cubebol	-	-	0.3	-	0.3	0.5
1496	valencene	0.6	0.5	0.3	t	-	-
1500	α -muurolene	-	-	-	-	-	0.4
1500	β -himachalene	-	t	0.1	-	0.1	-
1504	cuparene	-	t	0.1	t	-	-
1508	germacrene A	-	-	-	-	-	0.2
1506	(Z)- α -bisabolene	-	-	0.1	t	-	-
1512	α -alaskene	0.4	0.3	0.2	0.2	0.4	-
1513	γ -cadinene	-	-	-	-	-	1.4
1514	cubebol	0.8	0.7	0.4	1.2	0.3	-
1521	trans-calamenene	0.5	0.2	0.2	0.5	0.2	-
1522	δ -cadinene	0.5	0.3	0.3	0.6	0.2	1.6
1532	γ -cuparene	0.3	0.2	0.2	0.5	-	-
1537	α -cadinene	-	-	-	-	-	0.3
1548	elemol	-	-	-	-	-	1.2
1559	germacrene B	-	-	-	-	-	3.6
1561	(E)-nerolidol	-	-	-	-	-	0.1
1574	germacrene D-4-ol	-	-	-	-	-	4.0
1582	caryophyllene oxide	-	-	-	-	-	0.3
1589	allo-cedrol	1.7	2.0	1.9	2.4	1.4	-
1594	ethyl decanoate	-	-	-	-	-	0.3
1600	cedrol	25.5	29.3	25.4	27.5	22.6	-

KI	Compound	<i>J. excelsa</i>					<i>polycarpus</i>
		Kritchim Bulgaria	Lemos Greece	Eskisehir Turkey	Cyprus	Afqa, Lebanon	El Njass Lebanon
1608	humulene epoxide II	t	t	t	t	t	-
1608	β-oplophenone	t	t	t	t	t	0.4
1618	junenol	-	-	-	-	-	0.5
1622	sesquiterp. 67,134,174,202	-	-	-	-	-	1.0
1627	1-epi-cubenol	0.8	0.7	0.5	0.7	t	-
1630	γ-eudesmol	-	-	-	-	-	03
1632	β-acorenol	t	0.1	0.1	0.2	-	-
1638	epi-α-cadinol	t	t	t	t	-	0.9
1640	epi-α-muurolol	t	t	t	t	-	0.8
1644	α-muurolol	-	-	-	-	-	0.3
1645	cubenol	t	t	0.1	t	-	-
1649	β-eudesmol	-	-	-	-	-	1.1
1652	α-eudesmol	-	-	-	-	-	1.0
1653	α-cadinol	t	t	t	t	-	-
1661	sesquiterpene 85,57,41,238	1.1	0.9	1.0	1.2	1.3	-
1668	β-atlantone	0.7	0.5	0.6	0.7	0.8	-
1688	shyobunol	-	-	-	-	-	3.7
1700	eudesm-7(11)-en-4-ol	-	-	-	-	-	0.3
1713	cedroxyde	-	-	0.1	t	-	-
1792	β-eudesmol acetate	-	-	-	-	-	0.2
1987	manoyl oxide	t	0.2	0.1	t	-	0.6
2055	abietatriene	t	0.4	t	t	t	t
2087	abietadiene	-	-	-	-	0.2	0.1
2181	sandaracopimarinal	-	-	-	-	-	0.1
2298	4-epi-abietal	0.2	0.2	0.2	t	t	0.7
2401	abietol	-	-	-	-	-	0.1

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Variation in cedrol (% total oil) among individual trees in populations of *J. excelsa*. Note that the Cyprus value for cedrol is from bulked leaves from 5 trees.

Bulgaria	22.1	24.4	24.4	26.0	28.2
Greece	18.8	23.0	28.0	35.8	
Eskisehir	11.3	22.1	27.6		
Afqa, Lebanon	14.7	18.2	25.1		
Cyprus	27.5				

Table 3. Variation in cedrol (% total oil) among individual trees in populations of *J. polycarpus* and *J. p. var. turcomanica*.

<i>J. polycarpus</i> var. <i>polycarpus</i>					
Armenia	26.0	30.2	34.3		
Azerbaijan, hi cedrol	24.8	29.1	30.0	33.1	34.4
Azerbaijan, low cedrol	0.0	0.01	0.01	0.02	0.03
El Njass, Lebanon	0.0	0.01	0.01	0.01	
<i>J. polycarpus</i> var. <i>turcomanica</i>					
Kopet Mtns., Turkmenistan	0.1	0.1	0.2		
Fasa, Iran (introgressed)	0.0	0.1	1.1	10.1*	13.8*
*hybrid or introgressed from <i>J. seravschanica</i> ?					

Table 4. Leaf essential oils from populations of *J. polycarpus*, (El) Njass, Lebanon, *Adams 14158-14161*; Armenia, *Adams 8761-8763*, Azerbaijan, high cedrol, *Adams 14162, 14165, 14167, 14168, 14171*, low cedrol, *Adams 14163, 14164, 14166, 14169, 14170*; *turco* - *J. p. var. turcomanica Adams 8758-90*, Turkmenistan; *serav* - *J. seravschanica, Adams 8483-85*, Pakistan; *excel* - *J. excelsa, Adams 9433-35*, Eskisehir, Turkey. Components from cedarwood oils are highlighted in italics.

KI	Compound	<i>turco.</i>	<i>J. polycarpus</i> var. <i>polycarpus</i>				<i>excelsa</i>	<i>serav.</i>
		Turkm lo ced	Njass lo ced	Azer lo ced	Azer hi ced	Armenia hi ced	Turkey hi ced	Pakist hi ced
926	tricyclene	0.1	0.1	0.2	0.1	0.1	0.2	0.1
931	α -thujene	t	t	-	t	t	-	0.4
939	α-pinene	45.0	55.6	62.4	45.5	39.9	41.7	19.9
953	α -fenchene	0.1	0.2	0.1	0.1	t	0.3	0.1
953	camphene	0.2	0.3	0.4	0.3	0.2	0.2	0.2
957	thuja-2,4(10)-diene	t	0.1	0.1	t	t	0.1	-
961	verbenene	t	-	-	-	t	t	t
976	sabinene	0.3	t	0.2	0.2	0.4	0.1	0.5
980	β -pinene	0.8	0.8	0.9	0.7	0.5	0.7	0.9
991	myrcene	1.9	1.5	2.5	2.0	1.3	1.2	27.4
1005	α -phellandrene	-	t	t	t	-	0.1	0.1
1011	δ-3-carene	t	2.1	2.3	0.4	t	5.3	0.8
1018	α -terpinene	t	t	0.1	t	t	0.1	0.1
1026	p-cymene	0.2	0.2	0.2	0.1	0.2	0.6	0.6
1031	limonene	1.0	1.0	1.1	1.4	1.0	1.2	1.4
1031	β -phellandrene	0.8	1.0	1.0	0.9	0.4	0.9	1.3
1050	(E)- β -ocimene	-	-	t	t	-	t	t
1062	γ -terpinene	0.4	0.3	0.5	0.4	0.3	0.5	1.0
1068	cis-sabinene hydrate	t	t	t	t	t	t	0.2
1088	terpinolene	0.8	0.8	1.2	0.8	0.6	1.1	0.8
1098	linalool*	t	0.4	0.5	0.5	0.1	-	0.8
1116	3-methyl butanoate, 3-methyl-3-butenyl-	0.1	t	0.1	0.2	-	t	-
1121	cis-p-menth-2-en-1-ol	-	-	-	-	-	0.1	-
1125	α -campholenal	0.2	0.2	0.4	0.3	0.3	0.5	0.1
1139	trans-pinocarveol	0.2	0.2	0.2	0.2	0.2	0.8	t
1140	cis-verbenol	t	0.1	0.1	0.1	t	0.2	-
1143	camphor	1.0	0.3	0.9	0.4	-	1.2	-
1143	trans-verbenol	-	0.4	0.8	0.5	1.1	-	0.3
1148	camphene hydrate	t	t	t	t	-	0.1	-
1159	p-mentha-1,5-dien-8-ol	0.1	t	0.2	0.1	t	0.1	t
1160	pinocarvone	-	t	0.1	0.1	-	-	-
1165	borneol	-	0.2	-	-	-	-	-
1172	cis-pinocamphone	-	-	-	-	t	0.2	-
1177	terpinen-4-ol	t	t	0.1	t	t	0.1	0.4
1179	naphthalene	0.6	-	-	-	0.2	0.1	-
1183	p-cymen-8-ol	t	t	t	t	t	0.1	-
1189	α -terpineol	t	t	0.1	t	t	t	t
1193	4Z-decenal	-	0.1	0.2	0.1	-	-	-
1195	myrtenol	-	0.1	0.2	t	-	-	-
1204	verbenone	0.1	0.1	0.2	0.1	0.2	0.2	t
1217	trans-carveol	t	0.1	0.2	0.1	t	0.2	-
1243	hexyl 3-methyl butanoate	0.4	t	0.2	t	0.2	-	t
1252	piperitone	-	-	-	-	-	0.1	-
1257	4Z-decen-1-ol	-	0.2	0.2	0.1	-	-	0.1
1285	bornyl acetate	0.3	0.3	0.5	0.3	0.4	0.4	0.4
1290	trans-sabinyl acetate	-	-	-	-	-	-	0.2
1315	(2E,4E)-decadienal	t	-	0.1	-	-	2.4	0.3
1319	(2E,4E)-decadienol	-	-	0.1	-	-	-	-

		<i>turco.</i>	<i>J. polycarpus</i> var. <i>polycarpus</i>				<i>excelsa</i>	<i>serav.</i>
KI	Compound	Turkm lo ced	Njass lo ced	Azer lo ced	Azer hi ced	Armenia hi ced	Turkey hi ced	Pakist hi ced
1320	phenolic 149,91,77,164	-	-	0.1	-	-	-	-
1339	δ-elemene	0.1	0.2	0.1	t	t	-	t
1376	α-copaene	0.1	0.1	t	t	-	-	t
1382	hexyl n-hexanoate	0.1	-	0.2	0.1	0.2	-	0.2
1383	β-bourbonene	-	0.1	t	t	-	0.1	-
1389	β-elemene	0.2	0.2	-	t	-	-	t
1389	β-cubebene	t	-	-	-	0.1	0.1	-
1409	α-cedrene	0.1	-	-	0.7	1.0	0.8	0.6
1413	β-funebrene	-	-	-	0.7	1.5	0.7	0.7
1418	(E)-caryophyllene	0.6	0.7	0.4	-	0.8	-	0.3
1418	β-cedrene	t	-	-	0.6	0.2	0.5	0.2
1429	cis-thujopsene	-	-	-	0.4	0.4	0.3	0.2
1434	γ-elemene	t	0.2	-	-	-	-	-
1446	cis-muurolo-3,5-diene	t	-	-	0.1	-	0.2	-
1449	trans-sibirene	-	-	t	-	-	-	-
1454	α-humulene	0.2	0.1	t	-	-	0.1	t
1458	(E)-β-farnesene	-	-	-	0.2	0.3	0.2	0.2
1461	cis-muurolo-1(6),4-diene	0.2	-	t	0.1	0.1	-	t
1466	β-acoradiene	-	-	-	0.1	0.2	0.1	t
1473	trans-cadina-1(6),4-diene	-	t	t	0.1	-	0.2	-
1477	γ-muurolo-1,4-diene	0.3	0.2	0.1	-	-	-	t
1480	germacrene D	1.3	1.1	0.8	0.3	0.6	0.6	0.3
1489	β-selinene	-	0.2	-	-	-	-	-
1491	trans-murrolo-4(14),5-diene	0.2	-	0.3	0.3	-	0.2	0.2
1493	epi-cubebol	0.5	0.5	-	0.1	-	0.3	-
1498	β-alaskene	-	-	-	0.1	-	-	-
1494	bicyclogermacrene	-	-	-	-	0.3	-	t
1499	α-muurolo-1,4-diene	0.7	0.4	0.3	-	-	0.1	0.2
1502	cuparene	-	-	-	-	-	0.1	t
1503	germacrene A	0.2	0.1	-	-	-	-	-
1505	α-cuprenene	-	-	-	0.1	-	-	-
1509	β-bisabolene	-	-	-	t	t	0.1	t
1513	α-alaskene	-	-	-	0.5	0.1	0.2	0.3
1513	γ-cadinene	1.7	1.4	1.2	-	1.0	-	0.7
1513	cubebol	-	-	-	0.6	-	0.4	-
1524	δ-cadinene	2.8	1.6	1.4	0.4	0.8	0.5	0.8
1532	(E)-γ-bisabolene	-	-	-	-	0.3	0.2	-
1532	γ-cuprenene	-	-	-	0.2	-	0.2	t
1537	α-cadinene	0.4	0.3	0.2	-	-	-	0.1
1549	elemol	0.7	1.2	1.3	0.4	0.4	-	0.9
1556	germacrene B	2.8	3.6	1.9	0.8	1.6	-	0.7
1561	(E)-nerolidol	-	0.1	0.1	-	-	-	-
1574	germacrene D-4-ol	8.9	4.0	3.7	1.0	1.5	-	2.9
1582	caryophyllene oxide	-	0.3	t	-	-	-	-
1587	allo-cedrol	-	-	-	1.9	2.3	1.9	1.2
1594	ethyl dodecanoate	-	0.3	-	-	-	-	-
1596	cedrol	0.2	-	-	27.2	30.3	25.4	22.7
1606	β-oplophenone	0.4	0.4	0.2	-	-	t	-
1618	junenol	-	0.5	0.2	-	-	-	-
1622	sesquiterp. 67,134,174,202	-	1.0	-	-	-	-	-
1627	1-epi-cubenol	-	-	-	-	-	0.5	-
1628	sesquiterp. 43,119,161,204	-	-	0.3	0.6	-	-	-
1630	γ-eudesmol	-	0.3	t	-	0.4	-	-
1638	epi-α-cadinol	1.4	0.8	0.8	0.2	0.2	t	0.4
1640	epi-α-muurolo-1,4-diene	1.4	0.9	0.7	0.1	0.3	t	0.4

		<i>turco.</i>	<i>J. polycarpus</i> var. <i>polycarpus</i>				<i>excelsa</i>	<i>serav.</i>
KI	Compound	Turkm lo ced	Njass lo ced	Azer lo ced	Azer hi ced	Armenia hi ced	Turkey hi ced	Pakist hi ced
1645	α -muurolol	0.5	0.3	0.2	t	t	t	0.1
1649	β -eudesmol	0.2	0.8	0.4	0.1	t	-	0.2
1652	α -eudesmol	t	1.0	0.9	0.2	0.2	-	0.2
1653	α-cadinol	3.6	1.1	0.8	0.2	0.5	t	1.0
1661	sesquiterpene 85,57,41,238	t	-	-	-	-	1.0	-
1663	β -atlantone	-	-	-	-	-	0.6	-
1666	bulnesol	0.2	-	-	-	0.3	-	0.2
1666	(2E,4E)-decadienol	-	-	-	-	-	0.6	-
1688	shyobunol	2.1	3.7	2.9	1.0	1.2	-	1.6
1700	eudsm-7(11)-en-4-ol	-	0.3	0.1	t	-	-	-
1789	8- α -acetoxyelemol	0.1	-	-	-	-	-	t
1792	β -eudesmol acetate		0.2	0.1	t			
1989	manoyl oxide	-	0.6	0.2	0.3	0.4	0.1	0.2
2054	abietatriene	t	t	0.2	0.2	t	0.1	t
2080	abietadiene	0.7	0.1	1.5	1.0	0.8	0.5	0.3
2147	abieta-8(14),13(15)-diene	t	-	-	-	-	-	-
2184	sandaracopimarinal	0.1	0.1	t	t	-	-	-
2288	4-epi-abietal	1.9	0.7	0.9	0.9	1.5	0.2	1.2
2312	abieta-7,13-dien-3-one		-	0.2	0.1			
2331	trans-ferruginol		-	0.1	0.1			
2401	abietol		0.1	0.1	0.1			

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 5. Comparison of major components by total oil vs. oil minus cedarwood oil components for *J. polycarpus*, hi-cedrol populations. Total concentration (%) of cedarwood oil components: Azer hi ced: 31.5%, correction factor = 1.315; Armenia hi ced: 35.7%; correction factor = 1.357.

		<i>turco.</i>	<i>J. polycarpus</i> var. <i>polycarpus</i>			
KI	Compound	Turkm lo ced	Njass lo ced	Azer lo ced	Azer hi ced	Armenia hi ced
	concentration as % total oil					
939	α -pinene	45.0	55.6	62.4	45.5	39.9
1524	δ -cadinene)	2.8	1.6	1.4	0.4	0.8
1556	germacrene B	2.8	3.6	1.9	0.8	1.6
1574	germacrene D-4-ol	8.9	4.0	3.7	1.0	1.5
1652	α -eudesmol	t	1.0	0.9	0.2	0.2
1688	shyobunol	2.1	3.7	2.9	1.0	1.2
	concentration without cedrol, etc.					
939	α-pinene	45.0	55.6	62.4	59.8	54.1
1524	δ -cadinene	2.8	1.6	1.4	0.5	1.1
1556	germacrene B	2.8	3.6	1.9	1.1	2.2
1574	germacrene D-4-ol	8.9	4.0	3.7	1.3	2.0
1652	α -eudesmol	t	1.0	0.9	0.3	0.3
1688	shyobunol	2.1	3.7	2.9	1.3	1.6

Taxonomy of the Texas sunflower (*Helianthus praecox*) Asteraceae

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ABSTRACT

Helianthus praecox, known only from Texas, is treated as having three intergrading infraspecific taxa, these often treated as subspecies by various workers: var. *praecox*; var. *runyonii* (Heiser) B.L. Turner; and var. **hirtus** (Heiser) B.L. Turner, **stat. nov.** A review of the nomenclature is provided along with a key to the varieties, and a map showing their distribution. Published on-line www.phytologia.org *Phytologia* 96(2): 107-109 (April 1, 2014). ISSN 030319430

KEY WORDS: Asteraceae, *Helianthus praecox*, Texas

Schilling (2006) has provided a detailed taxonomic overview of the *Helianthus praecox* complex, treating this as comprised of three infraspecific categories: subsp. *praecox*, subsp. *runyonii* and subsp. *hirtus*, noting that they “appear to form a grade between *H. debilis* and *H. petiolaris*.” He also provided a perplexing key to the complex, made difficult because the several taxa appear to intergrade, hence my treatment of the infraspecific units as but varieties. I have studied all of the taxa anew and provide a key to the taxa concerned, along with a map showing their distributions.

HELIANTHUS PRAECOX Engelm. & Gray, Boston J. Nat Hist. 5: 221. 1847

Key to varieties

1. Outer involucral bracts mostly 8-11 mm long; apices of inner pales minutely ciliate, w/o tufted hairs; heads mostly 3-5 cm across the extended rays; peduncles mostly 5-15 cm long; coastal dunes of southeastern Texas (e Brazoria, Galveston and Chambers Cos.)var. **praecox**
1. Outer involucral bracts mostly 12-16 mm long; apices of inner pales tufted with white hairs; heads mostly 5-7 cm across the extended rays; peduncles mostly 5-15 cm long; southern Texas...**(2)**
2. Plants mostly 0.3-0.6 m high; mid-stems and petioles to some extent pubescent with spreading hairs 1-2 mm long; peduncles mostly 30-40 cm long; ray florets mostly 15-21; Carrizo Sands of Dimmit Co.....var. **hirtus**
2. Plants mostly 0.8-1.0 m high; mid-stems and petioles mostly pubescent with appressed hairs 0.2-1.0 mm long; peduncles mostly 20-30 cm long; ray florets mostly 11-15; throughout southern Texas.....var. **runyonii**

var. praecox

H. debilis ssp. *praecox* (Engelm. & Gray) Heiser

H. praecox ssp. *praecox*

The type of var. *praecox* was collected by Lindheimer on Galveston Island in Galveston Co., Texas, during the 1840s, presumably from dune sands. In my opinion, of the several varietal taxa, it is the

most distinct, as noted in the above key. It appears, however, to intergrade with var. *runyonii* in easternmost Brazoria Co. [S side of San Luis Bridge, Chavez s.n., 2 sheets (TEX)].

According to Heiser (1956), var. *praecox* co-occurs with the weedy, *H. annuus*, and he reports hybrids between the two at several localities; these I have not examined.

var. runyonii (Heiser) B.L. Turner, Phytologia 69:15. 1990.

H. debilis ssp. *runyonii* Heiser

H. praecox ssp. *runyonii* (Heiser) Heiser

This is a widespread, highly variable taxon, co-occurring with several species of *Helianthus*, with which it probably forms occasional hybrids. The variety intergrades with var. *praecox*, as noted above, and with var. *hirtus* in the vicinity of Carrizo Springs, Dimmit Co., as noted below.

var. hirtus (Heiser) B.L. Turner, **stat. nov.**

Based upon *H. debilis* ssp. *hirtus* Heiser, Madrono 13: 162. 1956.

H. praecox ssp. *hirtus* (Heiser) Heiser

Schilling (2006), following Rieseberg and Doyle (1989), treated *H. praecox* subsp. *hirtus* as a distinct subspecies, known only from a single population in Dimmit County. Their allozyme sample of the small population concerned was perhaps skewed; subsequent examination of the Carrizo Springs area by Holly and Nichols (unpubl.) in 1997 showed the existence of numerous populations (vouchers at TEX) composed of several thousand or more individuals, mostly from outcrops of the Carrizo Sands in the immediate vicinity of Carrizo Springs. More recent collections (2013) have been obtained from similar outcrops 14 mi NW of that city (Turner & Kos 13-2, TEX), and it is likely that the taxon occurs in closely adjacent Maverick Co. on similar outcrops.

Turner (1990) treated ssp. *hirtus* within the morphological rubric of var. *runyonii*, but the recent treatment of the complex by Shilling (2006), has occasioned a second look, prompting its recognition here.

ACKNOWLEDGEMENTS

I am grateful to Jana Kos for field and editorial assistance. Dot maps are largely based upon specimens on file at LL-TEX.

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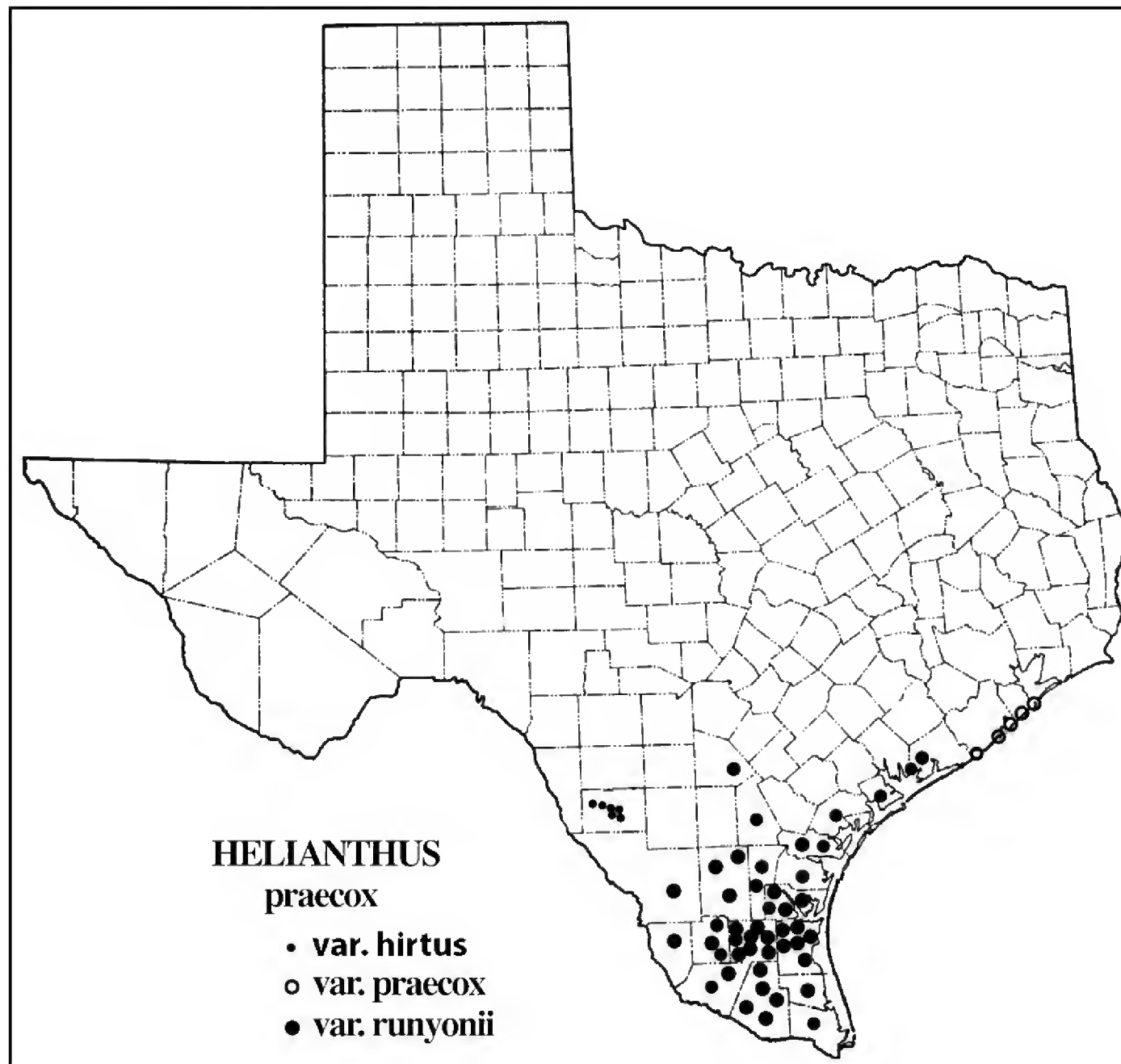


Fig. 1. Distribution of *Helianthus praecox*.

Geographic variation in the volatile leaf oils *J. phoenicea* var. *phoenicea* from throughout its range**Robert P. Adams**

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ABSTRACT

The compositions of the volatile leaf oils from 5 populations from throughout the range of *Juniperus phoenicea* var. *phoenicea* were analyzed. Two chemotypes were found: normal leaf oils and leaf oils containing cedarwood oil components. Except for the chemotypes (hi cedrol), the leaf oils of *J. phoenicea* are high in α -pinene (41.2 - 51.9%) and manoyl oxide (14.0 - 28.0%) with moderate amounts of α -pinene, myrcene, β -phellandrene and (E)-caryophyllene. Little geographic variation was found in the major components from Narbonne to Andorra, Zaragoza thence to El Peñón. The oil from the high cedrol plants at Grazalema seems quite different due to the presence of cedarwood oil components, but it is actually not very different, if one removes the heartwood terpenoids and re-normalizes the remaining terpenoids. Trees with high cedarwood oil had 16.4 - 31.9% cedrol and moderate amounts of other cedarwood oil components (eg., α - & β -cedrene, 2-epi-funebrene, cis-thujopsene, α - & β -alaskene, (E)- β -bisabolene, liguloxide, allo-cedrol). Published on-line www.phytologia.org *Phytologia* 96(2): 110-116 (April 1, 2014).

KEY WORDS: *Juniperus phoenicea* var. *phoenicea*, Cupressaceae, terpenes, leaf essential oil, geographic variation.

Recently, Adams et al. (2013) analyzed nrDNA and petN sequences for *J. phoenicea* L. (*sensu stricto*) from throughout the Mediterranean region (Fig. 1). They found *J. phoenicea* var. (or subsp.) *phoenicea* was restricted to Spain and France, whereas *J. phoenicea* var. *turbinata* (Guss.) Parl. (*J. turbinata* Guss.) were widely distributed from the Canary Islands to the Sinai (Fig. 4).

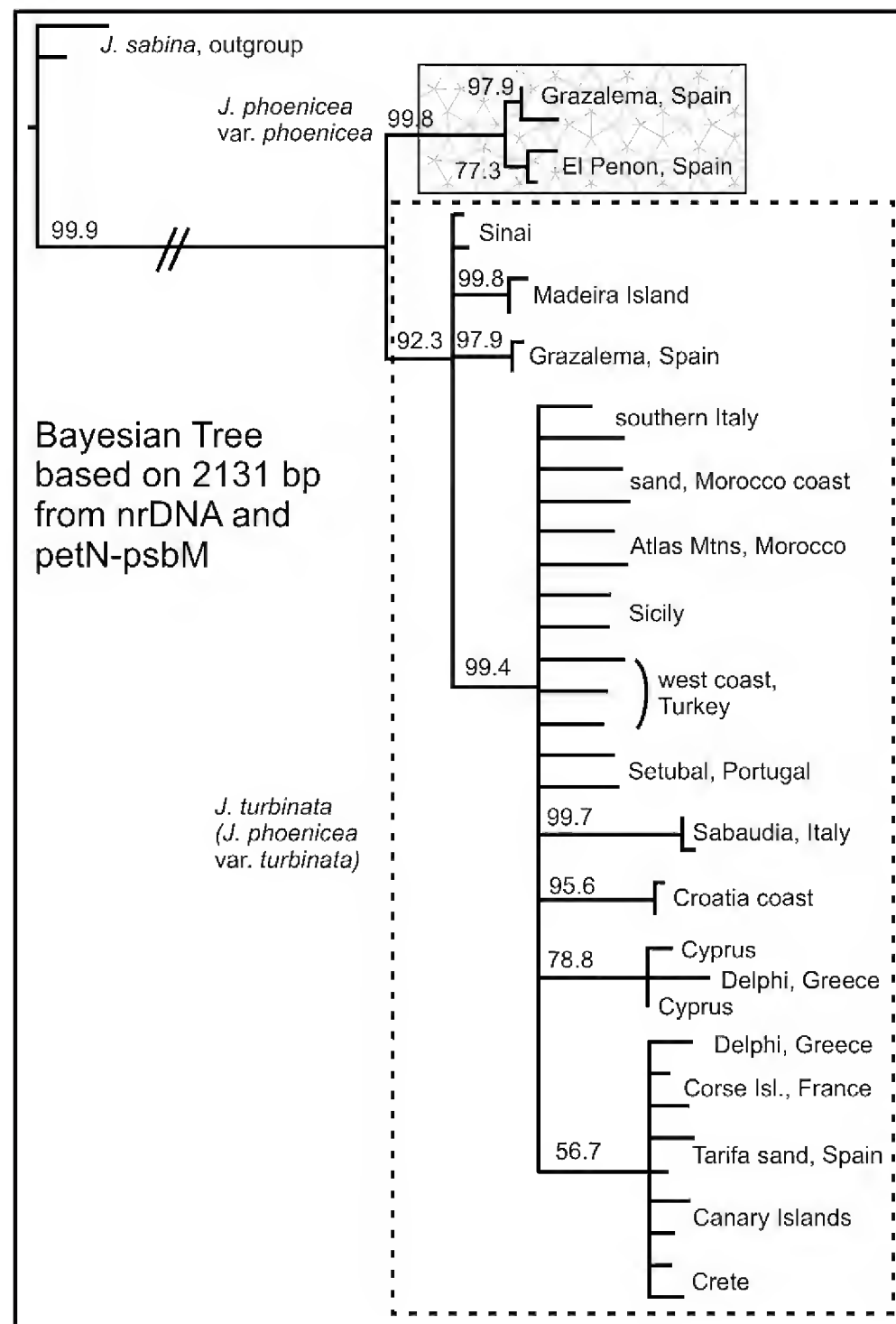
No differentiation was found between the typical Mediterranean and Canary Island populations, offering no support for the recognition of *J. phoenicea* subsp. *canariensis* (Guyot) Rivas-Martínez (Fig. 1). *Juniperus turbinata* appears to be widespread from Madeira - Canary Islands to the Sinai with few DNA differences among most populations. However, some populations (Grazalema, Madeira, Sinai, central Italy) displayed (Fig. 1) moderate amounts of divergence (3-4 mutations).

In a broad phylogenetic study of *Juniperus*, Adams and Schwarzbach (2013) found that *J. phoenicea* was not part of a clade of serrate-leaf junipers occurring in the western hemisphere, leading them to denote *J. phoenicea* as a 'pseudoserrate' juniper. In addition, they found *J. p.* var. *phoenicea* and var. *turbinata* to be as different in their DNA sequences as several other recognized species of *Juniperus*. This lends support for the recognition of *J. turbinata* Guss., as proposed by Lebreton and Pérez de Paz (2001) based largely on the concentration of prodelphinidin, a polymeric tannin. The prodelphinidin data suggested that *J. phoenicea* var. *phoenicea* was confined to the Iberian Peninsula with var. *turbinata* widespread throughout the Mediterranean region. Lebreton and Pérez de Paz (2001) found a clear

separation between *J. phoenicea* (Spain and France) and all other populations examined (*J. turbinata*).

Several studies have been made on the leaf terpenoids of *J. phoenicea*. San Feliciano and workers examined acidic diterpenes (San Feliciano et al., 1988; 1993). Incomplete analyses have been published on the volatile leaf oils of *J. phoenicea* from Egypt (Afifi et al., 1992), Saudi Arabia (Dawidar et al., 1991) and France (Tabacik and La Porte, 1971). See Adams, Barrero and Lara (1996) for a review of the early literature.

Adams, Barrero and Lara (1996) presented the first comprehensive analyses of the volatile leaf oils of *J. phoenicea*, *J. p.* subsp. *eu-mediterranea* and *J. p.* var. *turbinata*; they concluded that *J. p.* subsp. *eu-mediterranea* and var. *turbinata* were conspecific as their oils were nearly identical. More recently, Adams et al. (2009) presented complete analysis of the leaf oils of *J. phoenicea* (var. *turbinata*) from the Canary Islands and Madeira and compared these with oils from Morocco and Spain.



The purpose of the present study is to present a detailed analyses of the volatile leaf oils from populations of *J. phoenicea* var. *phoenicea* from throughout its ranges. (from Adams and Schwarzbach, 2013).

MATERIALS AND METHODS

Figure 2 shows the distributions of *J. phoenicea* var. *phoenicea* and populations sampled in this study.

Specimens used in this study: ***J. phoenicea* var. *phoenicea*:**

France, Narbonne, near St. Pierre sur Mere, 43° 10' 0.2" N, 3° 09' 57.6" E, 23 m, *J. Altarejos* 1-5, Baylor specs. Adams 14123-14127.

Andorra, Coll de Jou near Sant Julià de Lòria, 42° 26' 56.8" N, 1° 28' 04.6" E, 1426 m, *J. Altarejos* 6-10, Baylor specs. Adams 14128-14132.

Spain, Zaragoza, Montes de la Retuerta de Pina W of Bujaraloz, 41° 28' 59"N, 0° 19' 31.2"W, 317 m, *J. Altarejos* 11-15, Baylor specs. Adams 14133-14137.

Spain, El Peñón, 37° 35' 38" N, 3° 31' 22" W, 760 m, Adams 7077-7079,

Spain, Cádiz, Sierra de Graza lema, 36° 47' 51.5" N, 5° 24' 43.7"W, 835 m; *M. Arista* 1-5, Baylor specs. Adams 13813-13817.

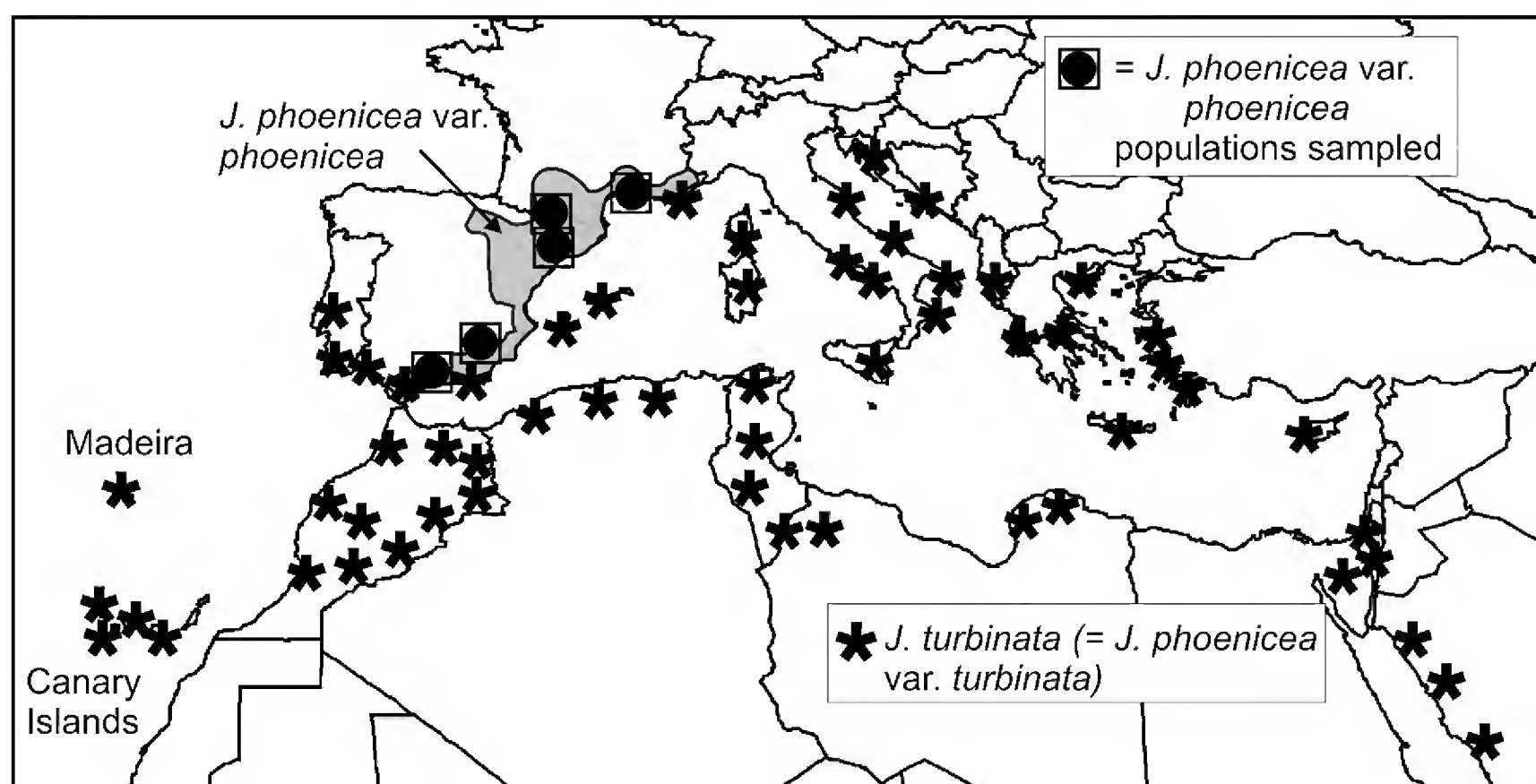


Figure 2. Distribution of *J. phoenicea* (adapted from Lebreton and Pérez de Paz, 2001, and Adams et al. 2010). Squares show the five populations of *J. phoenicea* sampled in the present terpene study.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 °C until analyzed. The extracted leaves were oven dried (100 °C, 48 h) for determination of oil yields.

Oils from 4 - 5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The composition of the volatile leaf oils of four of the five populations varied very little except for a chemotype (one tree) in the Zaragoza population that was high in cedrol and other cedarwood terpenoids (Table 1). However, all five trees sampled in the Grazalema population had the cedarwood chemotype and were high in cedrol (Table 1). The activation of the cedarwood oil pathway (in the leaf glands) reduces the concentrations of the non-heartwood components, as the terpene pool is siphoned off to produce cedarwood components in the leaf oil. There appears to be a single gene ('cedarwood synthase') that is turned on in the heartwood (or some associated tissue) that activates the cedarwood oil pathway (α - & β -cedrene, 2-epi-funebrene, cis-thujopsene, α - & β -alaskene, (E)- β -bisabolene, liguloxide, allo-cedrol, cedrol, widdrol, epi-cedrol, etc. (see Adams, 2014). Normally, this gene ('cedarwood synthase') is not active in *Juniperus* (and Cupressaceae) leaf oil glands. Most *Juniperus* species produce

two kinds of essential oils: leaf oils and heartwood oils and these oils have few components in common (Adams, 1991). *Juniperus phoenicea*, *J. excelsa*, *J. foetidissima*, *J. polycarpos*, and *J. seravschanica* have leaf oils that may contain significant amounts of the heartwood oil components (Adams and Hojjati, 2013). For example, Adams (1990) reported 4.4, 0.2, trace and 8.3% cedrol in the leaf oils from four trees of *J. foetidissima* from Greece. Whereas, Tunalier et al. (2004) reported 13.0 and 12.2% of cedrol and widdrol in the stem heartwood of *J. foetidissima* from Turkey. Ucar and Balaban (2002) analyzed the sapwood (white wood) of *J. excelsa*, Turkey, and reported the oil to contain 22.5% widdrol and 9.0% cedrol (these components are difficult to separate on non-polar columns and the mass spectra are nearly identical, so their identification is often problematic).

When *Juniperus* species contain heartwood components in the leaf oils, it is common to find chemical polymorphisms in cedrol (and associated heartwood terpenes) between trees. That is the case for trees from Zaragoza. Four trees had only the typical leaf oil components (Table 1) and their oil is very similar to nearby populations at Andorra and Narbonne, France (Table 1). However, one of 5 trees in the Zaragoza population had 31.9% cedrol and related compounds (Table 1) and thus, only 33.4% α -pinene. The oil of this tree, is quite similar to the hi cedrol Grazalema population that has 16.4% cedrol and 29.7% α -pinene (Table 1). It is interesting to compare cedrol + manoyl oxide for hi cedrol Zaragoza ($31.9+13.3 = 45.2$) vs. hi cedrol Grazalema ($16.4+32.9 = 49.3\%$).

Except for the cedarwood oil chemotypes (hi cedrol), the leaf oils of *J. phoenicea* are high in α -pinene and manoyl oxide with moderate amounts of α -pinene, myrcene, β -phellandrene and (E)-caryophyllene. Little geographic variation was found in the major components from Narbonne, Andorra, Zaragoza thence to El Peñón. The oil from the high cedrol plants at Grazalema seems quite different due to the presence of cedarwood oil components, but the oil is actually not very different, if one removes the heartwood terpenoids and re-normalizes the remaining terpenoids (Table 1).

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Table 1. Composition of the leaf oils of *J. phoenicea* (var. *phoenicea*): Narbonne, France; Andorra; El Peñón, Spain; Zaragoza (lo and hi cedrol), Spain; and hi cedrol, Grazalema, Spain. Those compounds that appear to distinguish taxa are in boldface. Cedarwood oil components are in italics. Values in parenthesis () for larger components of hi cedrol Zaragoza and hi cedrol Grazalema columns are corrected values, computed by correcting for cedarwood oil components.

AI	Compound	lo cedrol France	lo cedrol Andorra	lo cedrol El Peñón	lo cedrol Zaragoza	hi cedrol Zaragoza	hi cedrol Grazalema
921	tricyclene	0.1	0.1	0.1	0.1	0.1	t
932	α-pinene	42.4	42.9	41.2	51.9	33.4(45.7)	29.7(35.4)
945	α -fenchene	0.2	0.1	0.1	0.1	0.1	t
946	camphene	0.3	0.3	0.1	0.4	0.3	0.3
953	thuja-2,4-diene	t	0.1	0.1	0.1	0.1	t
961	verbenene	0.1	0.1	0.3	0.1	0.1	t
969	sabinene	t	t	0.1	t	t	t
974	β -pinene	1.7	1.7	2.1	2.0	1.2(1.6)	1.2(1.4)
988	myrcene	2.7	2.6	3.2	2.8	1.9(2.6)	2.3(2.7)
1001	δ -2-carene	t	t	0.1	t	t	t
1002	α -phellandrene	0.3	0.2	0.7	0.2	0.2	t
1008	δ -3-carene	2.0	1.3	1.5	t	t	t
1014	α -terpinene	t	t	0.1	t	t	t
1020	p-cymene	0.3	0.3	0.4	0.3	0.2	0.6
1024	limonene	0.6	0.6	0.6	0.5	0.4	0.4
1025	β-phellandrene	2.0	1.9	4.9	1.8	1.1(1.5)	0.6(0.7)
1054	γ -terpinene	0.2	0.2	0.2	0.2	0.2	0.9
1069	cis-linalool oxide	0.2	0.2	t	0.1	t	t
1086	terpinolene	0.7	0.6	0.7	0.6	0.4	0.4
1095	linalool	0.7	0.3	1.0	0.6	0.5	0.2
1118	cis-p-menth-2-en-1-ol	t	t	0.2	t	t	-
1122	α -campholenal	0.1	0.2	0.2	0.2	0.1	t
1135	trans-pinocarveol	0.1	0.2	0.3	0.4	0.2	t
1139	C ₁₀ OH, 41,55,81,95,152	-	-	1.4	-	-	-
1140	trans-verbenol	-	-	-	-	-	0.2
1141	camphor	0.4	0.5	-	0.5	0.3	-
1144	neo-isopulegol	0.3	0.4	t	0.4	0.3	t
1158	trans-pinocamphone	t	t	0.1	t	t	-
1165	borneol	0.2	0.3	0.6	0.3	0.1	-
1172	cis-pinocamphone	0.1	0.1	0.2	0.2	0.1	-
1174	terpinen-4-ol	t	t	0.1	t	t	t
1178	naphthalene	0.3	0.1	t	t	t	-
1179	p-cymen-8-ol	t	t	0.1	t	t	-
1186	α-terpineol	0.8	0.5	2.3	0.5	0.4	t
1195	myrtenal/ myrtenol	t	0.1	0.1	0.2	0.1	-
1204	verbenone	t	0.2	0.2	0.2	0.1	-
1215	trans-carveol	0.1	0.1	0.1	0.2	t	-
1223	citronellol	0.2	0.2	0.5	0.4	0.1	-
1249	piperitone	t	t	0.2	t	t	-
1255	(4Z)-decenol	0.1	0.1	0.2	0.1	t	t
1315	(E,E)-2,4-decadienal	t	0.5	0.3	0.2	t	-
1335	δ -elemene	0.1	0.1	t	0.2	0.1	t
1387	β -bourbonene	0.1	t	-	0.1	t	-
1389	β -elemene	0.4	0.1	0.1	0.3	0.1	-
1400	β -longipinene	t	0.4	t	0.4	0.1	t
<i>1410</i>	<i>α-cedrene</i>	-	-	-	-	<i>0.9</i>	<i>1.0</i>

AI	Compound	lo cedrol France	lo cedrol Andorra	lo cedrol El Peñón	lo cedrol Zaragoza	hi cedrol Zaragoza	hi cedrol Grazalema
1411	<i>2-epi-funebrene</i>	-	-	-	<i>t</i>	0.9	-
1417	(E)-caryophyllene	2.9	2.6	1.2	2.7	-	1.3(1.5)
1429	<i>cis-thujopsene</i>	-	-	-	-	0.4	0.3
1434	γ -elemene	0.1	t	t	0.1	-	-
1452	α -humulene	0.2	0.1	-	0.2	-	-
1454	(E)- β -farnesene	-	-	-	-	0.3	-
1478	γ -muurolene	t	t	-	0.1	0.6	-
1484	germacrene D	2.1	1.1	0.5	1.7	t	0.3
1484	allo-aromadendr-9-ene	t	t	-	0.1	-	-
1498	<i>β-alaskene</i>	-	-	-	-	0.1	-
1500	<i>β-himachalene</i>	-	-	-	-	0.1	-
1505	β -bisabolene	-	-	-	-	-	0.4
1509	C ₁₅ OH, <u>41</u> ,55,81,161,220	-	-	0.3	-	-	-
1512	<i>α-alaskene</i>	-	-	-	-	0.6	0.4
1513	γ -cadinene	0.1	0.1	0.1	0.1	t	t
1521	β -sesquiphellandrene	-	-	-	-	0.3	-
1522	δ -cadinene	0.3	0.2	0.2	0.3	t	0.2
1529	(E)- γ -bisabolene	-	-	-	-	0.2	-
1534	<i>liguloxide</i>	-	-	-	-	0.2	-
1535	C ₁₅ OH, <u>41</u> ,69,105,161,204	-	-	1.0	-	-	-
1541	C ₁₅ OH, <u>43</u> ,95,207,222	0.7	1.1	-	0.7	-	-
1548	elemol	0.5	0.9	1.8	1.8	0.5	0.5
1559	germacrene B	1.5	1.1	0.6	1.9	0.9	0.2
1561	(E)-nerolidol	0.1	0.1	t	0.1	t	-
1574	germacrene-D-4-ol	0.1	0.1	0.2	0.1	t	-
1582	caryophyllene oxide	0.7	1.1	1.0	1.2	0.5	-
1589	<i>allo-cedrol</i>	-	-	-	-	1.4	1.1
1600	<i>cedrol</i>	0.2	0.1	-	0.7	31.9	16.4
1625	C ₁₅ OH, <u>43</u> ,119,161,220	0.3	0.5	0.4	0.6	t	-
1630	γ -eudesmol	t	t	0.2	t	t	t
1632	<i>α-acoreanol</i>	-	-	-	-	0.4	-
1638	epi- α -cadinol	t	t	0.2	t	t	t
1638	epi- α -muurolol	t	t	0.1	t	t	t
1649	β -eudesmol	0.2	0.4	0.4	0.6	0.2	-
1652	α -eudesmol	0.4	0.6	0.3	0.7	0.3	t
1652	α -cadinol	-	-	0.3	-	-	t
1687	eudesma-4(15),7-dien-1- β -ol	-	-	0.1	-	-	-
1688	shyobunol	0.8	1.4	1.5	1.1	0.5	0.5
1715	(2Z,6E)-farnesol	t	t	1.2	0.2	t	-
1968	sandaracopimara-8(14), 15-diene	t	t	0.1	t	t	0.2
1978	manoyl oxide	28.0	25.4	22.0	14.0	13.3(18.2)	32.9(39.2)
2009	epi-13-manoyl oxide	t	t	0.1	t	t	0.2
2055	abietatriene	0.3	0.3	0.1	0.2	0.2	0.4
2087	abietadiene	0.1	0.2	0.1	0.1	t	t
2298	4-epi-abietal	0.2	0.4	0.2	0.4	0.2	0.2
2314	trans-totarol	0.9	1.2	0.2	0.4	0.5	1.9
2331	trans-ferruginol	0.1	0.1	t	t	t	0.3
	total % cedarwood cpds.	0.2	0.1	0.0	0.7	36.7	19.2

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Three new species of *Stevia* (Asteraceae: Eupatorieae) from northern Coahuila

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ABSTRACT

Three novelties of *Stevia* from Mexico, are proposed: **S. burroana** B.L. Turner, **sp. nov.**, **S. totalcoana** B.L. Turner, **sp. nov.** and **S. vacana** B.L. Turner, **sp. nov.**; the former from Coahuila relates to *S. ovata* var. *expansa*; the latter two from Vera Cruz and Coahuila relate to *S. tephra*. Type photographs of the several taxa are provided, including maps showing their distribution (along with those taxa to which they are most closely related). Published on-line **www.phytologia.org** *Phytologia* 96(2): 117-123 (April 1, 2014). ISSN 030319430

KEY WORDS: Asteraceae, Eupatorieae, *Stevia*, *S. ovata*, *S. tephra*, Mexico, Coahuila

The genus **Stevia** contains ca 200 species, 100 or more native to Mexico, many of these described (Soejima et al. 2001; Turner 2013; etc.) after the seminal work of (Grashoff 1972, 1974); its specious nature is largely occasioned by asexual reproduction compounded by chromosomal variability and hybridization, as well documented by Japanese workers (Soejima, A., T. Yahara and K. Watanabe 2001; Watanabe et al. 2001). No doubt additional novel taxa will come to the fore as more hinterland areas are collected.

STEVIA BURROANA B.L. Turner, **sp. nov.** **Fig. 1**

Suffruticose rhizomatous herbs or shrublets, up to 1.5 m high. **Leaves** mostly opposite, 4-10 cm long, 1.5-4.0 cm wide; petioles 2-10 mm long, passing into the blades; blades ovate, 3-nervate from near the base, glabrous above and below, or nearly so, weakly serrate, the margins pubescent. **Capitulescence** a terminal cymose panicle, 3-6 cm high, and as wide, the ultimate peduncles, 3-8 mm long, pubescent with minute glandular trichomes. **Heads** 6-7 mm high; involucre bracts beset with minute glandular trichomes and golden globules; apices acute. **Corollas** 5-6 mm long, glabrous, white to pink; tubes ca 2 mm long, passing into the throat; lobes ca 2 mm long, glabrous or nearly so. **Achenes** ca 5 mm long, brown, sparsely hispid; pappus a crown of lacerate scales ca 0.5 mm high.

TYPE: MEXICO. COAHUILA: Mpio. de Villa Acuna, “CA. 54 miles NW of Musquiz, in the RINCON DE MARIA of the limestone Sierra de la Encantada (Sa. De Santa Rosa),” 1960 m, “n-facing woodland-forest with oaks and *Psedotsuga*,” [etc.] 17 Sep 1999, *Henrickson 22531*. [with D.H. Riskind] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. COAHUILA: Mpio. de Villa Acuna, 1650 m, “near upper pila in canyon El Bonito,” 17 Sep 1977, *Riskind 2091* (TEX); Canyon El Toro, 18 Sep 1977, *Riskind 2127* (TEX); Canyon El Toro, 18 Sep 1977, *Riskind 2147* (TEX). “Rancho El Rincon, “on SW margin of Sierranias del Burro (part of Sierra del Carmen), ca 80 km SE of Big Bend National Park.” 1400-2100 m, 21 Aug 1991, *S.A. Ruiz 178* (TEX).

Because of the remarkable, minute, glandular trichomes on the ultimate peduncles and involucre of **S. burroana**, this novelty will key to **S. incognito** Grashoff in my key to the Mexican Stevias (Turner 1997), which it resembles not. Unfortunately, Grashoff (1972) did not possess material of **S. burroana** at the time of his study. On total characters, however, the novelty appears closest to the allopatric **S. ovata**

var. **expansa** Grashoff (including var. *texana* Grashoff of closely adjacent Big Bend region of Texas), which lacks glandular trichomes. Intermediates between the two allopatric taxa (Map 1) have not been noted.

The novelty is named for the Serranias del Burro, from which 4 of the 5 specimens have been collected.

STEVIA TOTALCOANA B.L. Turner, **sp. nov.** Fig. 2

Perennial herbs 30-60 cm high. **Mid-stems** 3 mm across, pubescent with crinkly hairs, the vestiture ca 0.5 mm high. **Leaves** (larger) 3-7 cm long, 1.0-1.5 cm wide; petioles 1-6 mm long; blades linear-lanceolate, mostly opposite below, alternate above, glabrous on both sides, passing into the petioles, the margins denticulate. **Capitulecence** a terminal cymose panicle 3-5 cm high, and as wide, the ultimate peduncles 1-5 mm long. **Heads** ca 1 cm high; the involucre bracts 7-9 mm long, glabrous to sparsely pubescent, their apices acute. **Corollas** white to pale pink, 5-6 mm long, glabrous or nearly so; tubes ca 2 mm long, passing into the throat, the lobes ca 2 mm long, sparsely pubescent beneath. **Achenes** brown, sparsely hispid above, 4 bearing a crown of lacerated scales, 0.5-1.0 mm high. 1(or 2) bearing bristles 3-6 mm long.

TYPE: **MEXICO. VERACRUZ: Mpio. Perote.** 2.1 mi SW of Totalco on western slope of rocky limestone ridge, ca 2350 m, 19 28 N, 97 22 W, 29 Oct 1983, *B.L. Turner 15435* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: all collected at the same locality as the type and on the same date: *Turner 15430* (TEX), *15437* (TEX).

I apparently collected the several sheets enumerated above because of the variability of their habit (perennial to suffrutescent herbs) and corolla color (white to pink). Except for such variation, the collections are very similar.

The several specimens cited above were initially identified as **S. tephra**, the type from Sierra de Pachuca, Hidalgo State. **Stevia totalcoana** differs from the latter in several characters, including head size, leaf size and shape, as well as being glabrous, as noted in the key below.

STEVIA VACANA B.L. Turner, **sp. nov.** Fig. 3

Perennial herbs, 45 cm high or more. **Mid-stems** purple, ca 2.5 mm across, pubescent with crinkly hairs, the vestiture ca 1 mm high. **Leaves** alternate, 4-5 cm long, 2-3 cm wide; petioles 2-5 mm long; blades broadly ovate, 3-nervate from near the base, passing into the petioles, upper and lower surfaces glabrous, except along the venation and margins, markedly glandular punctate. **Capitulescence** a terminal cymose panicle ca 15 cm high, and as wide, the ultimate peduncles 1-3 mm long, pubescent like the stems, with an understory of minute glandular hairs. **Heads** 10-12 mm high; involucre 7-9 mm long, pubescent with mostly upswept hairs, beset with amber globules. **Corollas** dark pink, 4-5 mm long, sparsely pubescent; tube ca 2 mm long, passing into the throat; lobes 1-2 mm long, sparsely pubescent beneath. **Achenes** black, 4-5 mm long, sparsely ciliate; pappus a crown of lacerate scales ca 0.5 mm high, a single achene (rarely 2) bearing 2-5 purple awns 3-5 mm long.

TYPE: **MEXICO. COAHUILA: Mpio de Ocampo,** Sierra del Pino, “ejido Acebuches, Canon La Vaca.” 28 15 N, 102 59 W, 1850 m.” Pinus-Juniperus forest, 2 Oct 2003, *M. A. Carranza c-4019* [with Ismael Ramirez]. (Holotype: TEX).

This novelty is readily separated from nearby populations of **S. ovata** var. **expansa** by a combination of characters: broadly ovate leaves, large heads (10-12 mm high vs 6-9 mm) and achenes 4-5 mm long. Indeed, when first examined I assigned the collection to that taxon (as did the original

identifier), to which it will begrudgingly key in my account of Mexican *Stevias* (Turner 1997). Reassessment of the latter complex has led to the recognition of the present novelty as a peripheral taxon related to the widespread *S. tephra* (Fig. 3), as envisioned by Grashoff (1972). So far as known the two taxa do not intergrade. A key to the *S. tephra* complex follows (cf. Fig 4):

1. Larger leaves linear-lanceolate, 4-6 cm long, widest near the middle, glabrous or nearly so; Mpio. Perote, Ver. ***S. totalcoana***
1. Leaves ovate to oval, 2-4 cm long, widest near base, mostly densely pubescent beneath (rarely not); north-central Mexico... **(2)**
2. Leaves broadly ovate, 4-6 cm long, mostly glabrous beneath; involucre bracts 7-9 mm long; Sierra del Pinos, Coa..... ***S. vacana***
2. Leaves oval to narrowly ovate, 2-4 cm long, mostly densely pubescent beneath; involucre bracts 5-7 mm long; widespread, mostly north-central Mexico..... ***S. tephra***

ACKNOWLEDGEMENTS

My editorial assistant, Jana Kos, read the paper, offering helpful suggestions. Dot maps are based upon specimens on file at LL-TEX, and those cited by Grashoff (1972).

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Fig. 1. *Stevia burroana*, (Holotype: TEX).

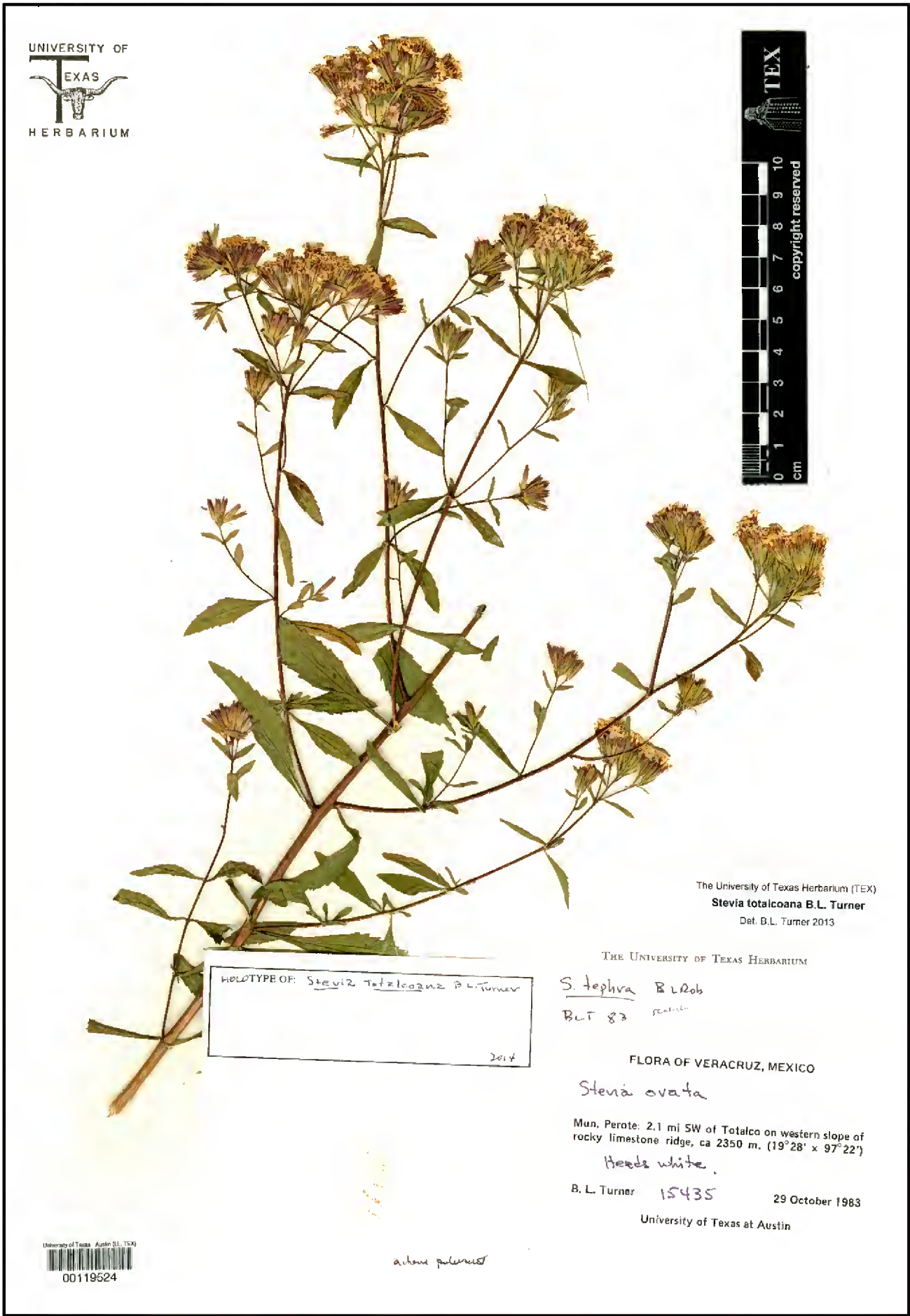


Fig. 2. *Stevia totalcoana* (Holotype: TEX).

Fig. 3. *Stevia vacana* (Holotype: TEX).

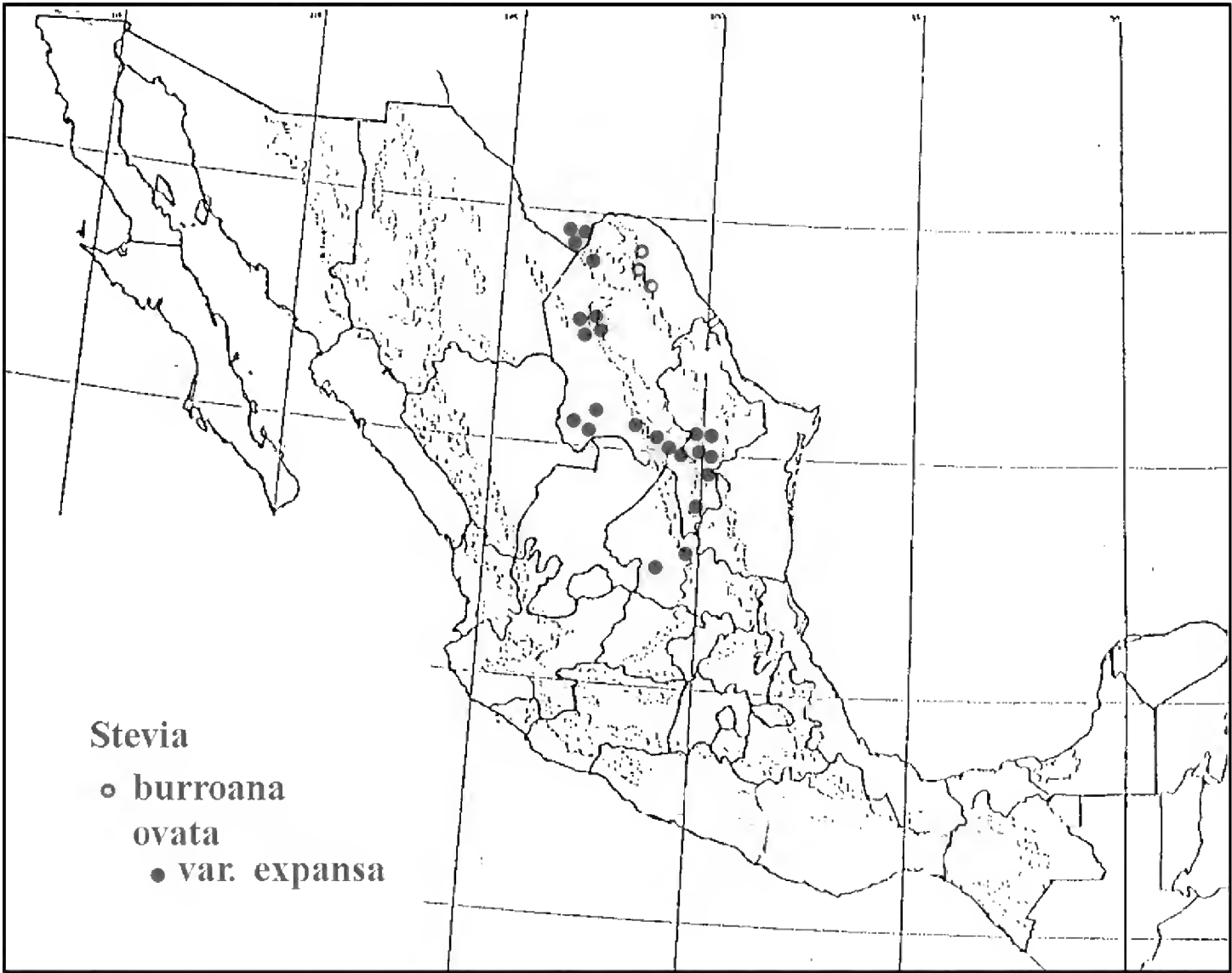


Fig. 4. Distribution of *Stevia burroana* and *S. ovata* var. *expansa*.

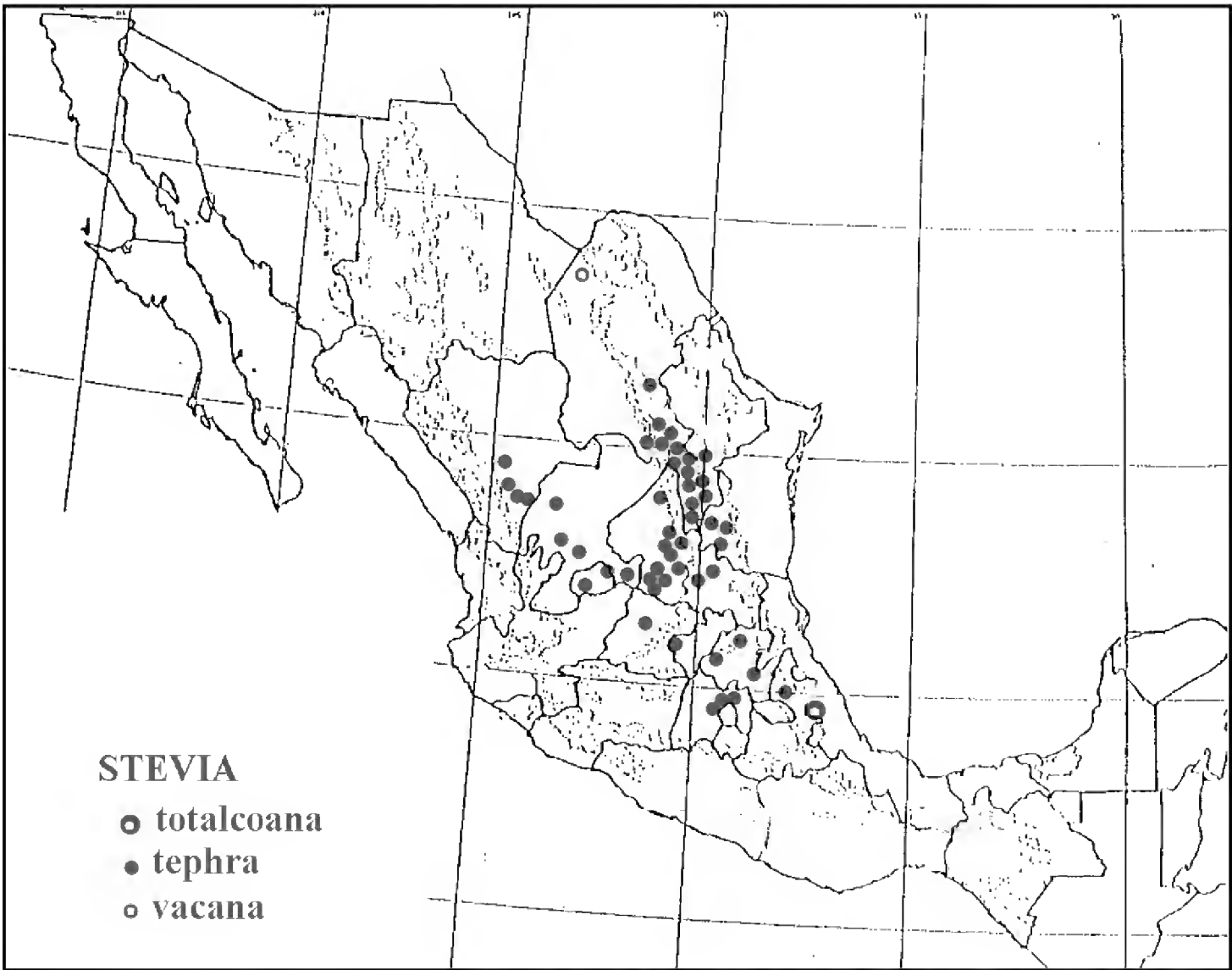


Fig. 5. Distribution of *S. totalcoana*, *S. vacana* and their closest relative, *S. tephra*.

Variation in *Juniperus communis* trees and shrubs from Bulgaria: analyses of nrDNA and cpDNA regions plus leaf essential oil.

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ABSTRACT

DNA sequence data from nrDNA plus four cp DNA regions (4315 bp) was congruent with leaf essential oils data. Based on leaf essential oils, DNA sequences and morphology, *J. sibirica*, Bulgaria, should be treated as *J. communis* var. *saxatilis*. Combined data on leaf essential oils, DNA sequences and morphology indicates that *J. pygmaea*, Bulgaria, should be treated as a shrubby form of *J. communis*: ***Juniperus communis* forma *pygmaea*** (K. Koch) R. P. Adams & A. N. Tashev, **comb. nov.** Published on-line www.phytologia.org *Phytologia* 96(2): 124-129 (April 1, 2014). ISSN 030319430.

KEY WORDS: *Juniperus communis* forma *pygmaea*, *Juniperus communis*, *J. sibirica*, *J. pygmaea*, Bulgaria, nrDNA, cpDNA sequences, leaf terpenes, taxonomy.

Assyov and Petrova (2012) recognized 6 native *Juniperus* species in the flora of Bulgaria: *J. communis* L., *J. excelsa* M.-Bieb., *J. oxycedrus* L., *J. pygmaea* K. Koch., *J. sabina* L. and *J. sibirica* Burgsd. Adams and Tashev (2012) reported that *J. oxycedrus* from Bulgaria was actually *J. deltoides* R. P. Adams, which grows from Italy eastward through Turkey. Of interest to the present work are the resolution and taxonomy of *J. communis*, *J. pygmaea* and *J. sibirica* (the latter two taxa treated as *J. c.* var. *saxatilis* by Adams, 2014 and Farjon, 2005, 2010). Of these 3 taxa, *J. communis* var. *communis*, grows as a small tree, whereas *J. pygmaea* and *J. sibirica* are small to spreading shrubs. They differ in their leaf morphology (Fig. 1) with *J. pygmaea* leaves being very similar to *J. communis*; those of *J. sibirica* differ by being shorter, curved and more appressed to the stem (Fig. 1). However, the leaves of the Bulgarian *J. sibirica* are quite similar to those of *J. communis* var. *saxatilis* Pall., as they are shorter and appressed as in the specimens from Mongolia and Norway (Fig. 2). Based on leaf morphology, *J. pygmaea* appears to be a shrub form of *J. communis* and *J. sibirica* appears to be *J. c.* var. *saxatilis*.

Recently, Adams and Tashev (2013) compared the leaf essential oils of *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria with the oils of *J. communis*, Sweden and *J. saxatilis*, Switzerland (Fig. 3). From their analysis, the oils do not ordinate *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria into separate groups, but they are generally interspersed (Fig. 3). There is a suggestion of a grouping of *J. sibirica* (oval, Fig. 3), but no clear clustering.



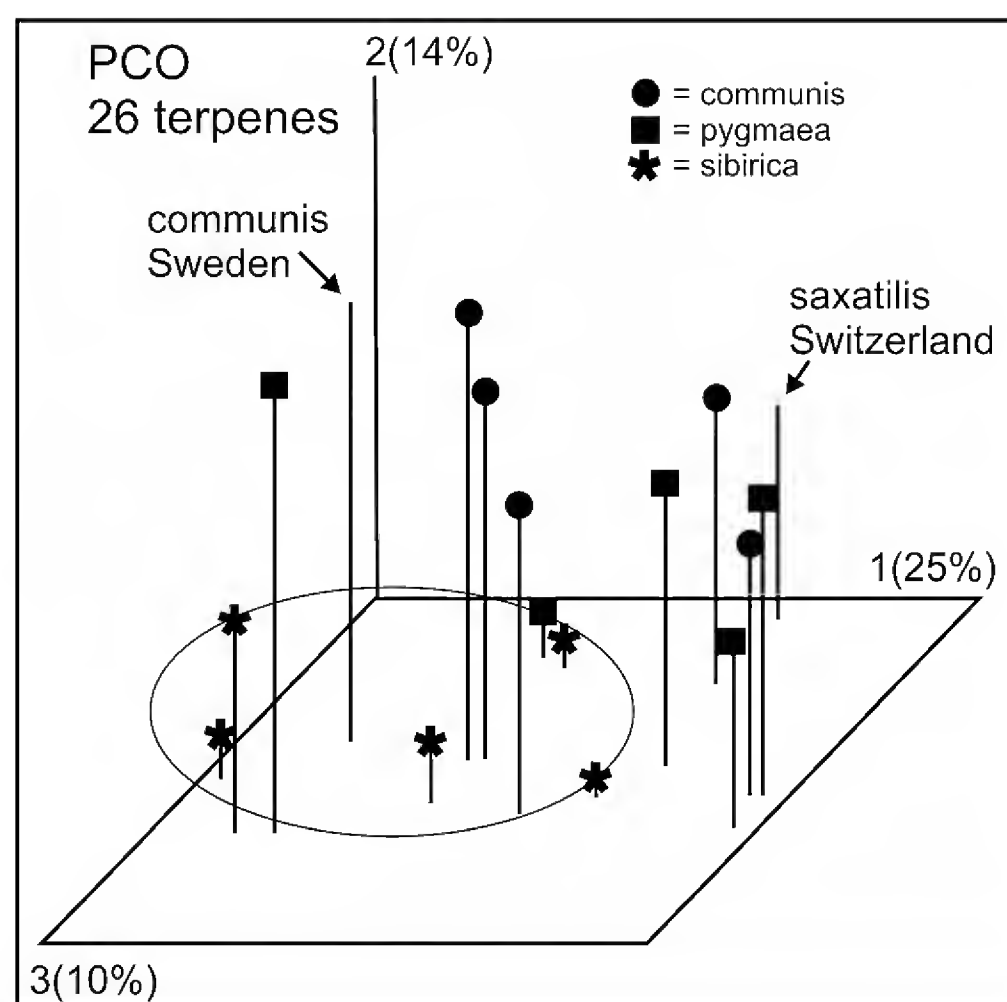
Figure 1. *J. communis*, *J. pygmaea* and *J. sibirica* specimens from Bulgaria.



Fig. 2. Specimens of *J. c. var. saxatilis* from Mongolia and Norway and var. *hemispherica* from Mt. Etna.

Figure 3. PCO ordination based on 26 leaf terpenes of *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria with exemplars of *J. communis*, Sweden and *J. c. var. saxatilis*, Switzerland.

The purpose of this study was to compare data from nrDNA and four cpDNA regions of *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria with other members of *Juniperus* sect. *Juniperus* from the eastern hemisphere to determine if these taxa differ in their DNA. Note that both *J. pygmaea* and *J. sibirica* were treated as synonyms of *J. saxatilis* by Adams (2014) and Farjon (2005), but to avoid confusion, the usage as per the Flora of Bulgaria (Assyov and Petrova (2012) is used in the present paper .



MATERIALS AND METHODS

Plant material - Bulgaria, *J. communis* var. *communis*, Adams 13730-31, 14058-60, Alex Tashev, 2012-JC1-5, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca*, 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m.

J. pygmaea K. Koch, Adams 13734-35, 14064-66, Alex Tashev, 2012-JP1-5, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies*, 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.

Juniperus sibirica Burgsd. (cf. *J. communis* var. *saxatilis*), Adams 13732-33, 14061-63, Alex Tashev, 2012-JS11-5, Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus*, *V. uliginosum*, *Ribes petraeum*, *Rubus idaeus*, *Calamagrostis arundinacea*, *Festuca valida* (Bulgarian endemic), 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.

Exemplar specimens: *J. communis* var. *communis*, Stockholm, Sweden, Adams 8167 (7846-7848); *J. communis* var. *saxatilis*, Switzerland, Adams 11164 (7618-7621). Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al. 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp-regions petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF yielded 4315 bp of data. The Bayesian consensus tree (Fig. 4) revealed that all the *sibirica* accessions are in a clade with *J. c.* var. *communis*, Sweden and *J. c.* var. *saxatilis*, Norway. Three of the *J. c.* var. *communis*, Bulgaria are in a well-supported clade, whereas the other two are in a clade with three *pygmaea* (Fig. 4). It is interesting that *J. c.* var. *saxatilis* is polyphyletic. Adams (2014) noted that *J. c.* var. *saxatilis*, from the Japan region, should probably be treated as *J. c.* var. *nipponica*. Two of the

pygmaea plants had mixed bases in their nrDNA suggesting they may be of hybrid origin. Notice that they group with *J. c.* var. *communis* and that the other three *pygmaea* are in a separate clade.

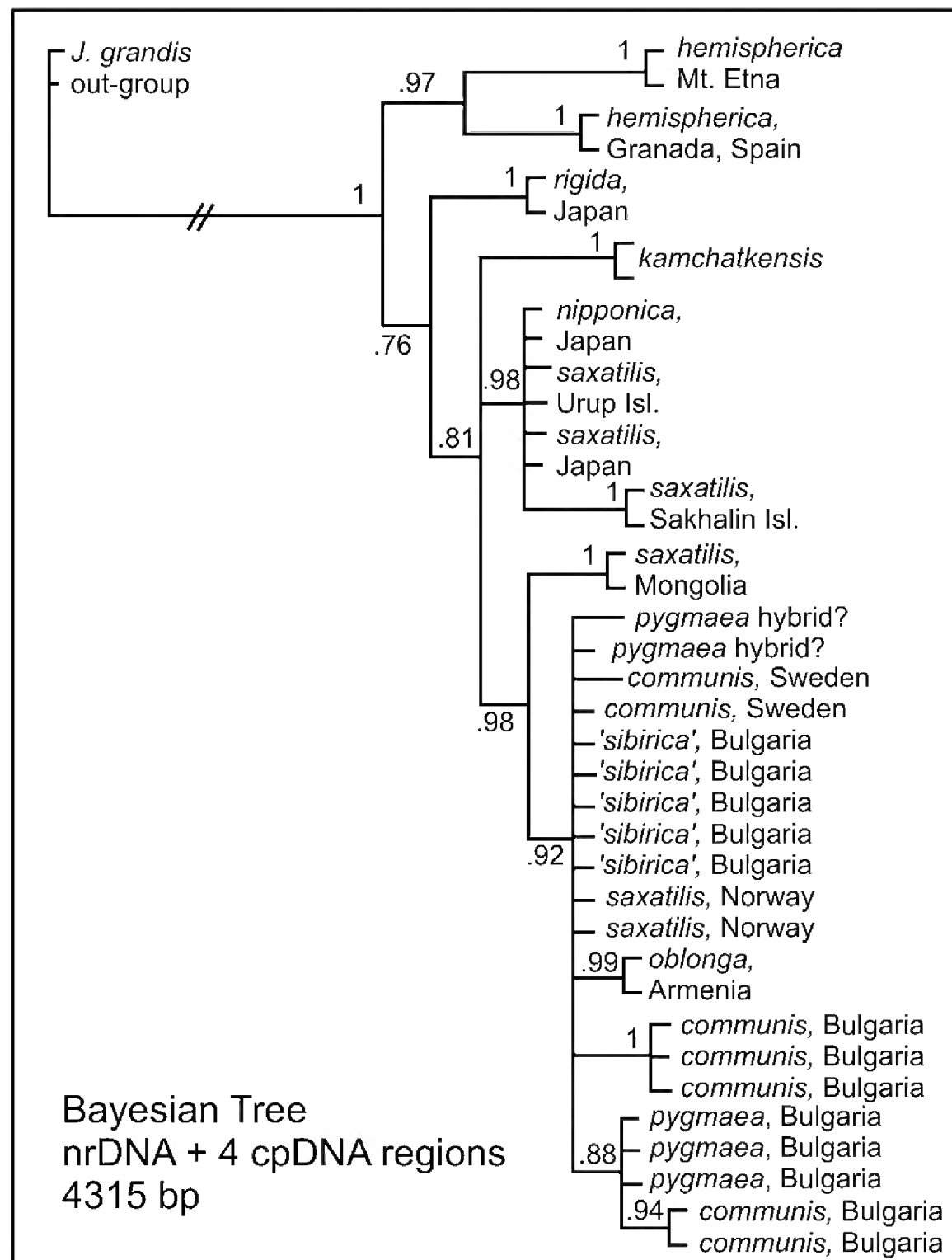


Figure 4. Bayesian tree of *Juniperus* sect. *Juniperus* taxa of the eastern hemisphere. Numbers at branch points are posterior probabilities. Two of the *pygmaea* plants had mixed bases in their nrDNA suggesting they may be of hybrid origin. Notice that they group with *J. c.* var. *communis* and the other three *pygmaea* are in a separate clade.

To examine the magnitude of the differences, a minimum spanning network was constructed (Fig. 5). *Juniperus communis*, eastern hemisphere, is divided into three groups: *J. communis*, Europe, *J. communis*, Japan and far east, and *J. c.* var. *hemispherica*, the latter divided among Mt. Etna, Sicily (type locality) and Sierra Nevada, Granada, Spain. All the samples from Bulgaria are tightly grouped with *J. communis* from Europe (Fig. 5). No variation was found among the five samples of *J. sibirica* and they have no differences from *J. communis* var. *communis* (type locality, Sweden), nor from *J. c.* var. *saxatilis* (Norway), although the *saxatilis* from Norway appears to only be a shrubby form of *J. communis* var. *communis*. Thus, based on leaf essential oils, DNA sequence data and morphology, *J. sibirica* of Bulgaria, should be treated as *J. communis* var. *saxatilis*.

The five samples of the Bulgarian *J. c.* var. *communis* (trees to shrubby trees) displayed two kinds of DNA. Two trees were like three shrubs of *J. pygmaea*, and the other three *communis* trees had DNA that was differed by 3 MEs from *J. communis* (Sweden), etc. (Fig. 5). The *J. pygmaea* samples were in two groups separated by 2 MEs (Fig. 5). This minor variation to be expected in natural populations.

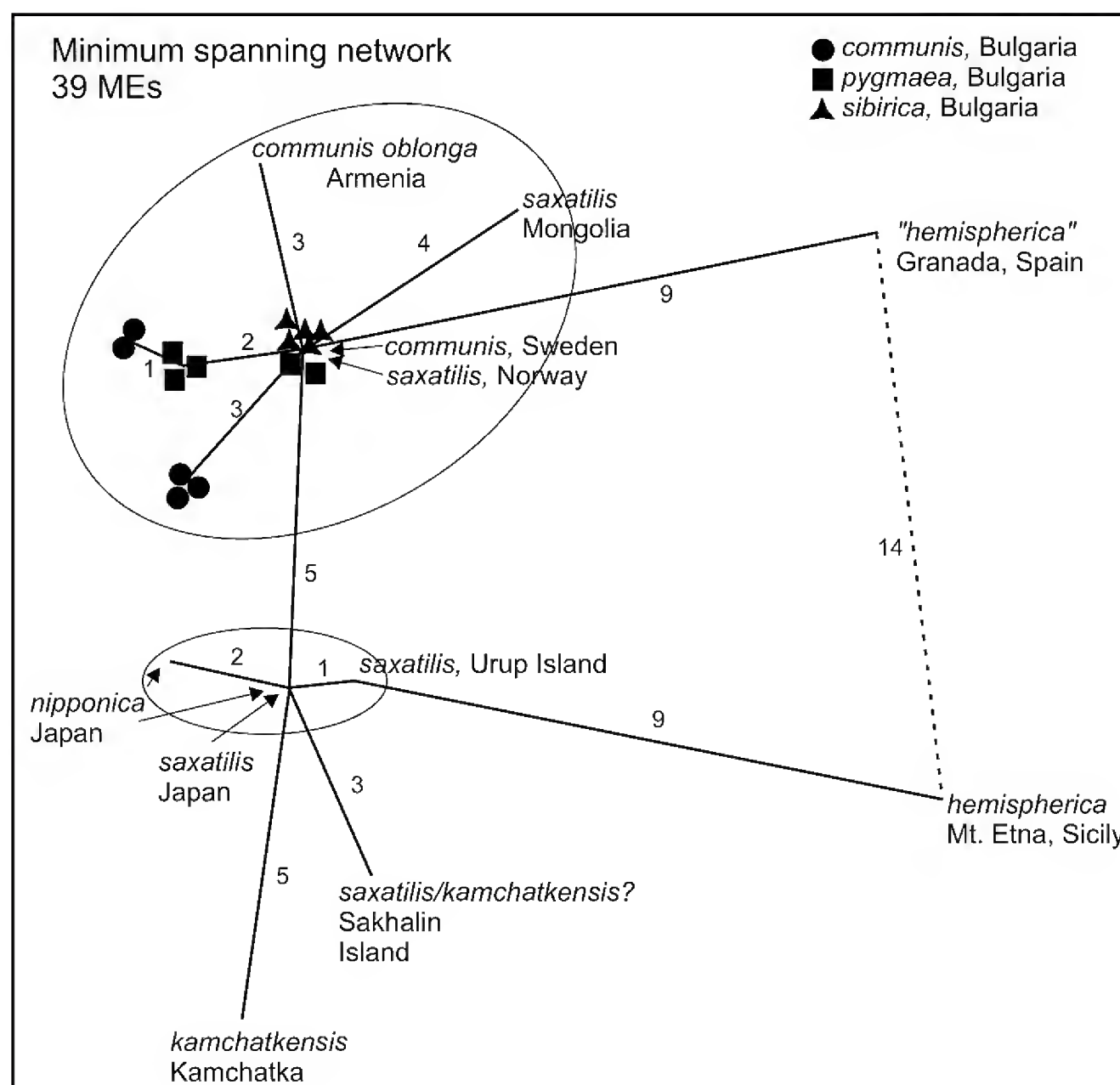


Figure 5. Minimum spanning network of *J. communis* and its varieties based on 39 MEs (mutational events = SNPs + indels). Numbers next to the links are the number of MEs. The dashed line is the second shortest link between the *hemispherica* taxa.

In short, based on leaf essential oils, DNA sequence data and morphology, *J. pygmaea* of Bulgaria, appears to be a shrubby form of *J. communis* var. *communis*, and not *J. c.* var. *saxatilis*, *sensu stricto* and should be recognized as but a form:

Juniperus communis* forma *pygmaea (K. Koch) R. P. Adams & A.N. Tashev, **comb. nov.**, Fig. 1 (center).

Basionym: *Juniperus pygmaea* K. Koch, Linnaea 22: 302 (1849). Type: unknown (coll. K. Koch 1843-44, B lost in fire) "Auf dem pontischen Hochgebirge der Gaue Hemschin und Pertakrek" (Pontic Mtns., southern side of the Black Sea).

J. communis subsp. *pygmaea* (K. Koch) Imkhan., Novosti Sist. Vyssh. Rast. 27: 10. 1990.

ACKNOWLEDGEMENTS

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**A naturally occurring hybrid between *Asclepias syriaca* L. and *A. purpurascens* L.
(Apocynaceae, Asclepiadoideae)**

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ABSTRACT

In 2012 a naturally occurring hybrid between *A. syriaca* L. and *A. purpurascens* L. was found growing along a roadside in Dent Co., MO. It was photographed and the flowers compared with those of its parent species. Hybridization between these two species has not been previously reported. Published on-line www.phytologia.org *Phytologia* 96(2): 130-134 (April 1, 2014). ISSN 030319430

KEY WORDS *Asclepias syriaca*, *Asclepias purpurascens*, Hybrid, Apocynaceae, Asclepiadoideae, Dent Co., Missouri

Hybridization seems to be rare between species of *Asclepias*, judging by the few reports available in the literature and the general failure of attempts to produce hybrids artificially (pers. obsv.). Charles Robertson (1887) noted that “the modifications of the floral structure of different species [of *Asclepias*] enable the plants to avoid competition for the same insects, or for the same parts of the same insects. Thus, bumble-bees have pollinia of *A. sullivantii* on their claws, of *A. verticillata* on their tarsal hairs, and of *A. longifolia* on the hairs of the ventral surface.” Variation in coronal structure is a way to prevent hybridization. Even so, over the years a few observant people have noticed milkweed plants growing naturally that had characters outside the normal parameters for the species it most resembled, and that such plants often seemed to be intermediate between two species growing fairly close to each other. Usually it was just a single plant. In some cases these plants were considered merely as anomalies, but in others they were described as new species, even when these single plants were recognized as probable hybrids. Vail (1904) described three new species of *Asclepias*. The first, *A. kansana*, from Riley Co. in east-central Kansas, was probably a natural hybrid between *A. syriaca* and *A. speciosa*. In my own field studies, I found that hybrids between those two species are fairly common in central Kansas where their ranges overlap, the hybrids there have the tomentum and erect, fewer-flowered umbels of *A. speciosa* with the shorter coronas of *A. syriaca*. Vail’s description is a good match for the hybrids I saw there. The other two she cited as possible hybrids of *A. syriaca*, one with *A. amplexicaulis*, which she named *A. intermedia*, and another with either *A. amplexicaulis* or *A. exaltata* which she named *A. bicknellii*. Unfortunately, Vail did not seem to realize that a single hybrid plant is not a new species until it forms part of a reproducing community of similar plants. Moreover, she presented no evidence that this was the case with the specimens she was describing. Churchill (1918) found a smooth-podded form of *A. syriaca* in Berkshire Co., MA, that he named *A. syriaca* forma *inermis*. Woodson (1926) cited a specimen collected in Marshall Co., IN as a possible hybrid between *A. syriaca* and *A. viridiflora*. It was included in a herbarium search in which he found 9 possible hybrids (including the two of Vail’s), none of them between the two species dealt with here. No hybridization between *A. syriaca* and *A. purpurascens* has been reported previously.

Brown (1906), reported on a natural hybrid between *Ceropegia sandersonii* and *C. similis*, which he named *C. hybrida*. Stephan Vogel (1960) reported on natural hybrids between *C. sandersonii* and *C. nilotica*. Broyles (2002) noted that “natural hybridization occurs throughout areas of sympatry for the

North American milkweeds, *Asclepias exaltata* and *A. syriaca*" even though the formation of F₁ hybrid seed is a rare event.

OBSERVATIONS & CONCLUSIONS

In the fall of 2012 in Dent Co., MO, Karen Diane Smith found what appeared to be plants of *A. syriaca* with smooth pods similar to those of *A. purpurascens* which was growing nearby. In Jun of 2013 an examination of the flowers showed them to be a probable hybrid between the two species. The cluster of plants maintained the tall habit (nearly 2 m) of *A. syriaca* with its multiple lateral umbels of pink flowers (normally on somewhat nodding pedicels), but the latter had fewer, erect flowers that were similar in size & shape to those of *A. purpurascens*. The anther wings were like those of *A. syriaca* but the pollinaria were similar to those of *A. purpurascens*. The leaves were intermediate between the two species. The plants also maintained the gemmiferous roots of *A. syriaca*, resulting in a cloned colony of multiple stems within about a 3 m length of ditch. Though the gynostegia of *A. syriaca* and *A. purpurascens* are similar, the hoods of the former are considerably shorter than those of the latter and this may contribute to the scarcity of hybridization between these two species. It seems probable that the flowers of this clone will be pollinated predominantly by pollinia from normal plants of *A. syriaca* and that these intermediate characters will eventually be absorbed back into the greater population. It is likely that interspecific hybridization may account for the variation that is often found within many of the species of *Asclepias*.

Seeds were collected from the hybrid plants in the fall of 2012 and stored over the winter at room temperature. The following spring the seeds were placed on moist sand in petri dishes and subjected to 30 days of 35° cold treatment. Returned to room temperature, germination was high (80%) and numerous seedlings resulted.

Finally, it should be noted that more recent workers have reported on the existence of natural hybrids among species of *Asclepias*, including *A. syriaca* (Broyles, 2002).

ACKNOWLEDGMENTS

I express my gratitude to Karen Diane Smith for calling my attention to this remarkable plant, and to Thomas J. Rosatti and Roy Gereau for reviewing the manuscript.

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Figure 1. *Asclepias syriaca* x *A. purpurascens*. Hybrid flowers. Inset shows the pollinarium.



Figure 2. *Asclepias syriaca* x *A. purpurascens*. Hybrid with smooth fruits.

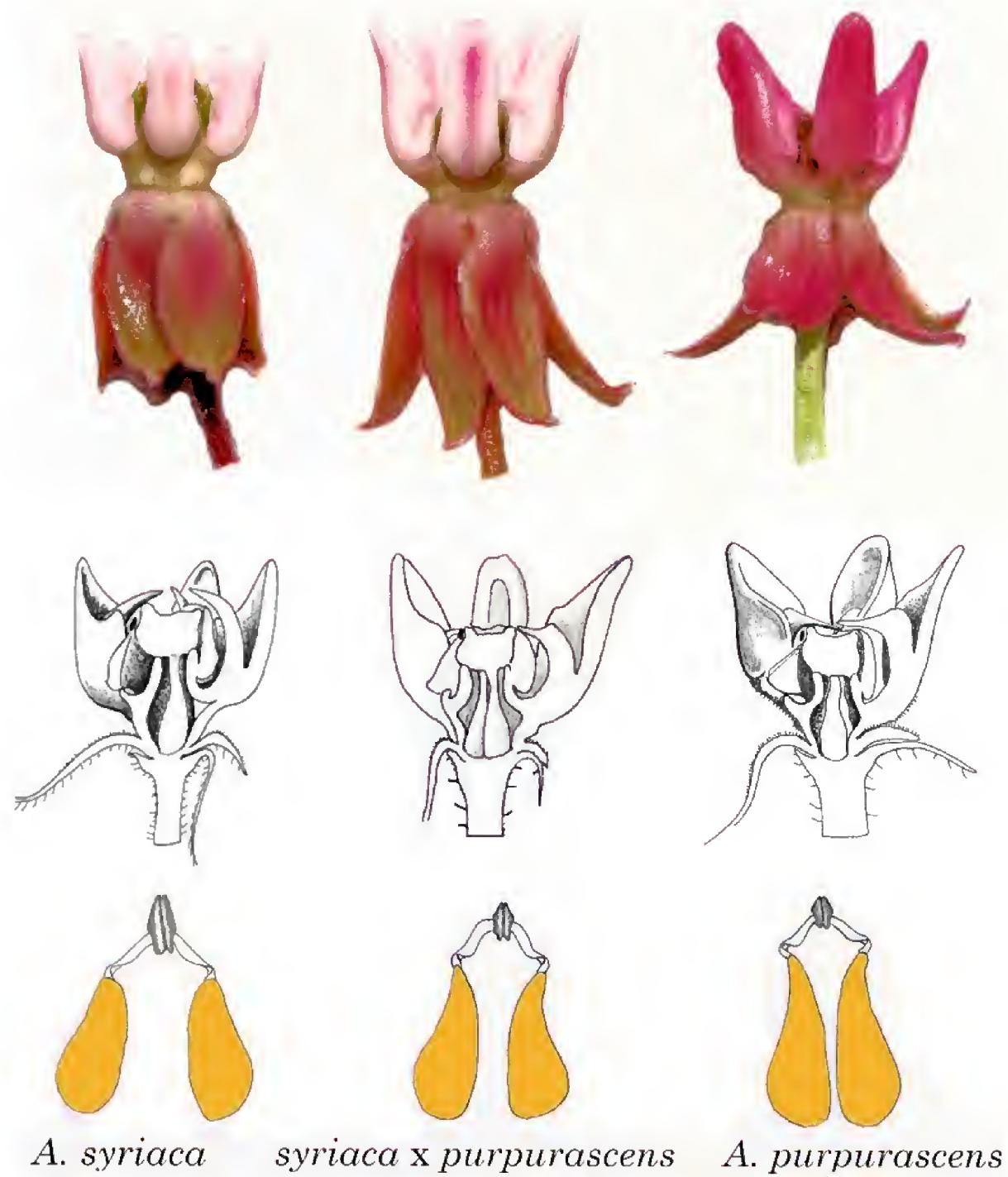


Figure 3. *Asclepias syriaca* x *A. purpurascens*. A comparison of the three flowers. Note in the flower diagrams and in the pollinaria, the hybrid flowers favor *A. purpurascens*, but in the preceding photos the habit of the plant favors *A. syriaca*.

The foliage terpenoids of *Callitropsis nootkatensis*: Comparison of oils from cultivated and natural trees

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ABSTRACT

Variation in the leaf terpenoids of *Callitropsis nootkatensis* was examined between cultivated and natural trees for four cases: **Case I.** Comparison of individual terpene concentrations and oil yields of Rennell Sound Pass trees grown in two sites (CLRS, JR) on Vancouver Island, BC revealed two significant (myrcene, α -terpinyl acetate) and one highly significant difference (terpinolene). No significant difference was found in % oil yield. PCO ordination showed that the CLRS and JR samples were interspersed, not separated by plantation site, suggesting the variation was mostly under genetic control rather than environmentally induced. **Case II.** ANOVA of oils from cultivated trees (CLRS site) compared to natural trees at John Day, OR showed that the concentrations of five of the six major monoterpenes were significantly or highly significantly different. However, % oil yield was not significantly different. PCO ordination revealed 3 chemotypes in which natural (John Day) trees were interspersed, implying that genetically controlled differences may be largely responsible for the ordination pattern. **Case III.** ANOVA of oils from cultivated trees at JR site compared to natural trees at Mt. Angeles, WA revealed two terpenes were highly significant different and another significant different in concentration. There was no significant difference in % oil yields. PCO showed the samples from cultivated and natural trees were mostly interspersed, pointing to genetic control rather than environmental factors for the pattern. **Case IV.** ANOVA comparing oils from cultivated trees (CLRS) verses natural trees at Mitkof Island, AK found no significant differences in any terpenoid or % oil yields. However, two chemotypes were found among the terpenoids in both cultivated and natural trees. The Mitkof Island case presents clear evidence that environmentally induced differences between oils from cultivated or natural trees has far less of an influence in comparison to chemotype (genetic) differences among individuals whether from cultivation, or natural populations of *C. nootkatensis*. Overall, in these four cases, it appears that the genetic component is much greater than the environmental component in determining both oil yields, and composition in the *C. nootkatensis* studied.

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KEY WORDS: *Callitropsis nootkatensis* (*Chamaecyparis nootkatensis*), terpenes, environment, Alaska yellow cedar.

The most complete, early analysis of the leaf oil of *Chamaecyparis nootkatensis* (treated as *Callitropsis nootkatensis* in this paper) was by Cheng and von Rudloff (1970). Subsequently, trans-totarol was isolated from *Chamaecyparis nootkatensis* (Constantine et al., 2001). Cool (2001) found new ent-daucane and acoranes from *xCupressocyparis leylandii*, but the compounds were not found in either of the putative parents (*Chamaecyparis nootkatensis*, *Cupressus macrocarpa*). Von Rudloff (1975) and Yatagai et al. (1985) published additional leaf oil compositional data based on one or a few individual trees. The most recent complete analysis of the volatile leaf oil of *Chamaecyparis nootkatensis* (as *Xanthocyparis nootkatensis*) is by Adams et al. 2007.

There does not appear to be a study of the genetic verses environment effects on the oil yields and terpenoid composition of *C. nootkatensis*. To examine these effects, four cases were chosen for this study. Case I. Trees grown at two sites on southern Vancouver Island (CLRS, Cowichan Lake Research Station, planted in 1988, 24 yr old and JR, Jordan River, planted in 1994, 18 yr old) were available for study and represented an unusual opportunity to examine the leaf oils from the same population growing in two different plots. Case II. Comparison of oils from natural trees at the Cedar Grove Botanical Area (near John Day, OR) compared to cultivated trees at CLRS (from cuttings taken at Cedar Grove). Case III. Comparison of oils from natural trees at Mt. Angeles, Olympic National Park, WA (ONP) compared to cultivated trees at JR. Case IV. Comparison of oils from natural trees at Mitkof Island, AK compared to cultivated trees at CLRS.

MATERIALS AND METHODS

Callitropsis nootkatensis plant materials - (samples taken from CLRS or JR sites, Nov. 2012):
 Rennell Sound Pass, at JR, Lab Acc. Adams 13701-13705: JR 225, 231, 233, 234, Rennell Sound Pass; Haida Gwaii, BC, 53° 22' N, 132° 18' W, 280m;
 Rennell Sound Pass, at CLRS, Lab Acc. Adams 13706-13709: CLRS 563, 564, 567, 569, 570, Rennell Sound Pass; Haida Gwaii, BC, 53° 22' N, 132° 18' W, 280m;
 John Day, cultivated at CLRS, Lab Acc. Adams 13664-13667, CLRS 343, 345, 346, 347, Cedar Grove Botanical Area (CGCA), John Day, OR, 44° 21' N, 119° 20' W, ca. 1730m;
 John Day, natural trees, Cedar Grove Botanical Area (CGCA), near John Day, OR, Adams 14112-14117, 14121-14122, ex Mark Corbet #1-8, 6 Oct 2013, 44° 20' 15.6" N; 119° 20' 17.1" W. elev. 1730 m, Malheur Co., OR.
 Mt. Angeles (lower slope), Olympic National Park (ONP), cultivated at JR site, Lab Acc. Adams 13673-13677, JR 312, 313, 314, 316, 317, Mt. Angeles, WA, 47° 59' N, 123° 28' W, 1405 m;
 Mt. Angeles (lower slope), Olympic National Park (ONP), natural trees, Hunter and Fairhall 1-6, 20 Oct. 2013, Lab Acc. Adams 14138 - 14143, Mt. Angeles, ONP, WA, 47° 58.96' N, 123° 27.99' W, elev. 1370 m.;
 Mitkof Island, AK, cultivated at CLRS site, Lab Acc. Adams 13710-13714: CLRS 374, 375, 377, 380, 381, Mitkof Isl, AK, 56° 49' N, 132° 57' W, ca. 30m;
 Mitkof Island, AK, natural trees, K. Dillman, 1-5, 23 Oct. 2013, Lab Acc. Adams 14149-14153, Mitkof Island, AK, 56° 48' 26" N; 132° 57' 28" W. elev. 15 m.
 Voucher specimens are preserved in vitro at CLRS and JR plantations, Vancouver Island, B. C. Voucher specimens for Cedar Grove Botanical Area, John Day, OR, Mt. Angeles, WA and Mitkof Island, AK are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Additional steam distillation tests for up to 48 h revealed that the 2 h distillation removed about 23% (of the total oil for 48 h), providing a correction factor of 4.34 (x 2h = 48 h total). The yields appeared to asymptote at about 104% of the 48 h total oil.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal Coordinate Ordination (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

Table 1. Comparison of site characteristics between JR and CLRS plantations on southern Vancouver Island, BC.

Site	Latitude	Longitude	Elevation m	MAT	MWMT	MCMT	TD	MAP	MSP
JR	48° 25' 28.0" N	124° 00' 55.9" W	105m	9.3	16	3.3	12.6	2465	364
CLRS	48° 49' 7.7" N	124° 07' 48.2" W	185m	8.9	17.2	1.9	15.3	2192	284

Site	SHM	SNOW	SI	SOILS	BEC variant	Site association
JR	44	0	32	glacial till/Podzolic sandy loam	CWhxm2	1
CLRS	61	100	20	glacial till/Podzolic gravelly sandy loam	CWhvm1	5

Table notes:

MAT -mean annual temperature; MWMT - mean temperature of the warmest month; MCMT - mean temperature of the coldest month; TD - Continentality (MWMT-MCMT); MAP - mean annual precipitation; MSP - mean summer precipitation; SHM - moisture index=(MWMT/(MSP/1000)), SNOW - mean height (m) at 50 years; SI - site index=productivity; BEC variant - xm2=dry maritime; vm1=very moist maritime; Site association - 1=zonal; 5=wetter and richer.

RESULTS AND DISCUSSION

Case I. Oils from Rennell Sound Pass trees grown in two plantations (CLRS and JR).

ANOVA of the terpenoids (as % total oil) plus total % oil yields from Rennell Sound Pass samples grown at CLRS and JR plantations revealed two significant (myrcene, α -terpinyl acetate) and

one highly significant (terpinolene) difference (Table 1). No significant difference was found in % oil yield. No significant differences were found among the major components (cf. α -pinene, δ -3-carene, limonene, β -phellandrene, Table 1). ANOVA of the terpenoids on a mg/g basis gave similar results with the same three monoterpenes being significantly different (Table 2).

To visualize the similarities using % data, a similarity matrix was assembled and factored. Six eigenroots of 24.7, 21.79, 16.6, 10.69, 9.79, and 6.52% accounted for 89.6% of the variation among the 9 samples. The large number of eigenroots (6) from such a small number of samples (9) indicates that the data set is highly variable. PCO shows the CLRS and JR samples are intermixed, not separated by plantation site (Fig. 1). An analysis using mg/g data for each terpenoid gave a similar ordination (Fig. 2).

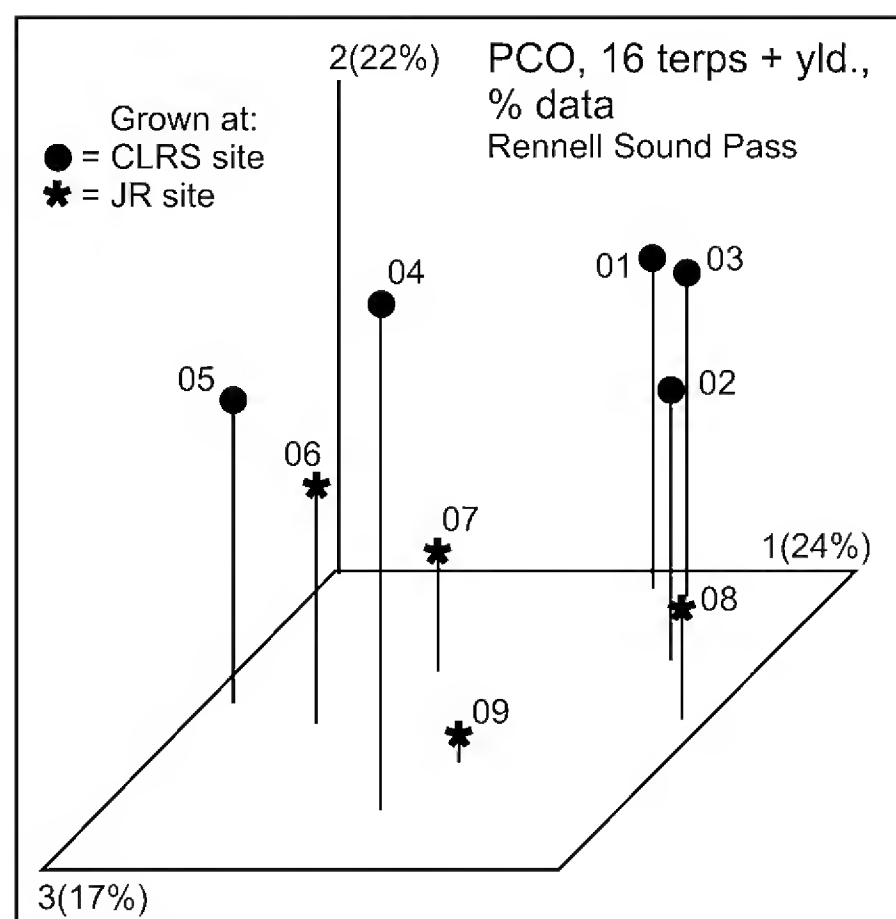


Figure 1. PCO using % total oil data, for Rennell Sound Pass trees grown at the CLRS and JR plantations.

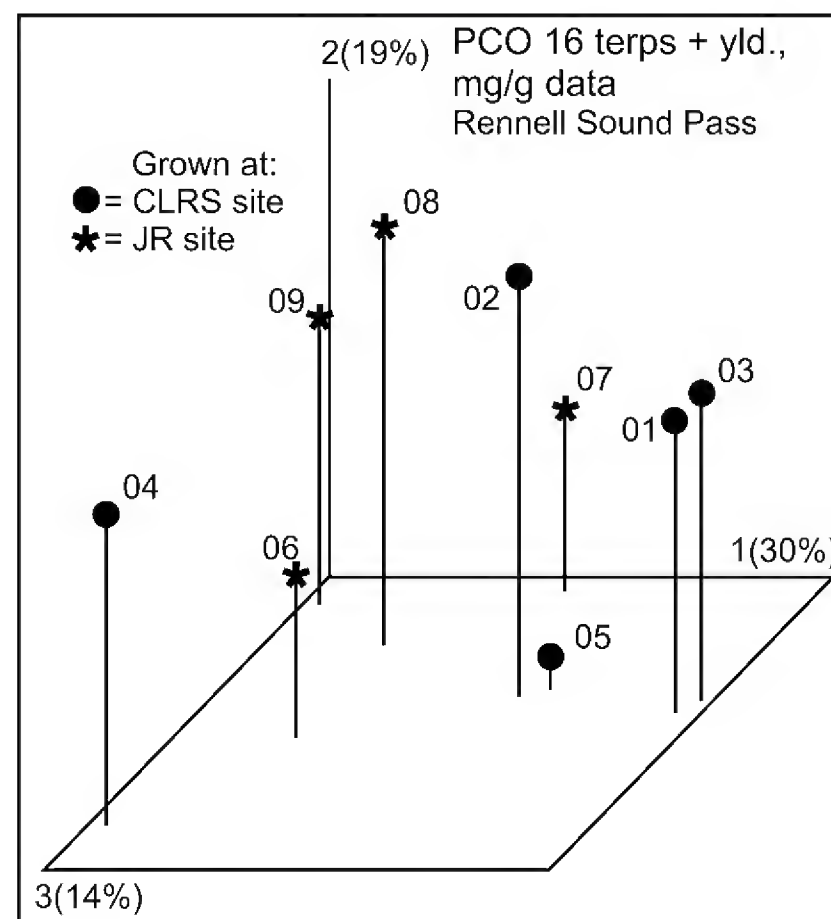


Figure 2. PCO using mg/g data, for Rennell Sound Pass trees grown at the CLRS and JR plantations.

Examination of the oils from trees at CLRS and JR (Table 3) shows there is considerable variation within the CLRS and JR samples. It seems likely that genetic differences among trees are the major factor in the terpene levels.

To examine the correlation among individual terpenes and % oil yield, PCA was performed on the correlation matrix of the 16 terpenoids (plus % oil yield). Three eigenroots accounted for 33.9, 26.0, and 17.9% (77.7% total) of the variance among the samples. Ordination of the terpenoids shows the patterns of correlation (Fig. 3).

Percent oil yield was negatively correlated with APNN (-0.54) and BPNN (-0.54) and positively correlated with LMNN (0.54) and BPHL (0.54). Many of the monoterpenes are highly correlated forming a

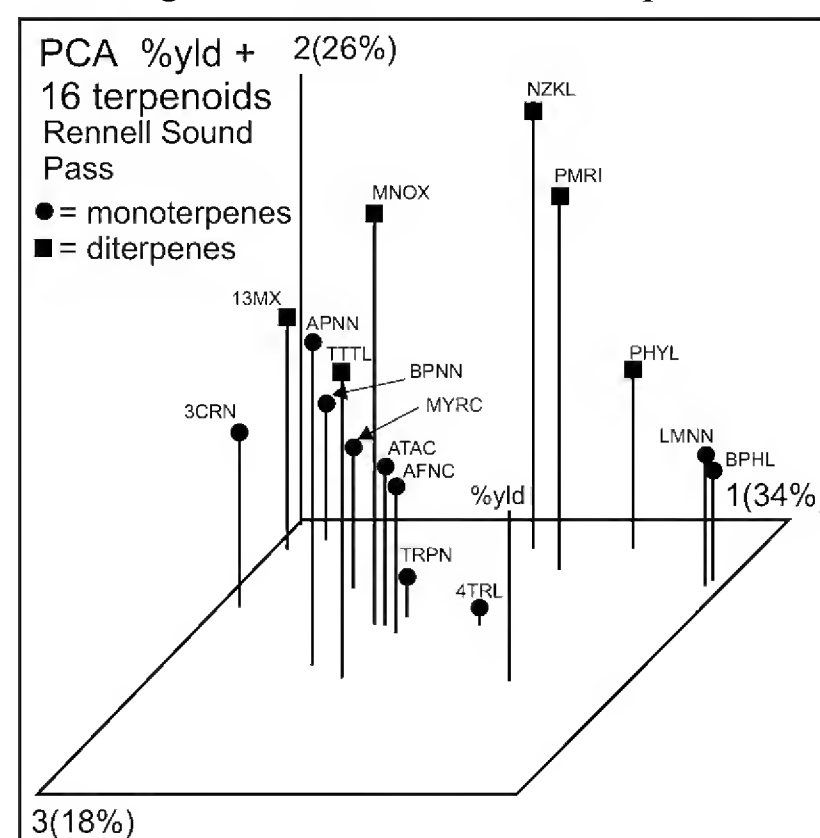


Figure 3. PCA ordination showing correlation between yield, monoterpenes and diterpenes.

group (Fig. 3, left). Limonene and β -phellandrene are highly negatively correlated with α - and β -pinene, 3-carene, myrcene (-0.86 to -0.51) and this results in their separation on the first principal component (Fig. 3.).

Interestingly, some of the diterpenes (13-epi-manool, trans-totarol, manool oxide) are correlated with the α - and β -pinene, 3-carene, myrcene group and other diterpenes (nezukol, iso-pimaradiene, and phyllocladanol) are correlated with limonene and β -phellandrene (Fig. 3).

In summary, the leaf oils of trees from seed samples collected from Runnels Sound Pass, Haida Gwaii, BC and grown at the CLRS and JR plantations showed little differences in their oils. Because the samples did not group by CLRS or JR site in PCO ordination, it appears that genetic differences played a much larger role in the determination of the terpenoid profiles than differences in the environment between the CLRS and JR sites.

Case II. Oils from naturally grown compared to cultivated trees: John Day, OR population.

Callitropsis nootkatensis has three isolated, inland populations, two in southern British Columbia, and another in eastern Oregon at the Cedar Grove Botanical Area (CGBA) near John Day, OR. The John Day population is interesting because it is very isolated and also very well defined in size. Cuttings were collected and rooted about 25 years ago and planted at the CLRS plantation on Vancouver Island. Samples of fresh foliage were collected Oct 2013 from 8 trees and steam distilled for comparison with oils from 4 trees cultivated at CLRS (collected Nov. 2012) to compare leaf oil yield and terpenoid composition.

ANOVA between John Day natural and cultivated trees (Table 4, % cpd data) revealed five of the six major monoterpenes' concentrations were significantly or highly significantly different (α -pinene, β -pinene, myrcene, limonene and β -phellandrene). Percent oil yield, although a little larger in the natural population was not significant. None of the diterpenes was significantly different.

To visualize clustering among individuals, a similarity matrix was assembled and factored. Seven eigenroots of 29.3, 19.5, 13.5, 8.8, 7.7, 5.3 and 4.6% accounted for 88.6% of the variation among the 12 samples. The large number of eigenroots (6) from such a small number of samples (12) indicates that the data set is highly variable. PCO shows (Fig. 4) three chemotypes: 1. low α -pinene, low δ -3-carene, high limonene/ β -phellandrene; 2. high α -pinene, high δ -3-carene, low limonene/ β -phellandrene; 3. high α -pinene, low-medium δ -3-carene, medium limonene/ β -phellandrene (Fig. 4 and Table 5). CLRS samples are found intermixed with the samples from natural trees in chemotypes 2 and 3 (Fig. 4), but not in chemotype 1.

Differences in the terpenoid composition between and within chemotypes are shown in Table 5. Nezuol is highly variable within chemotypes 1 and 3, ranging from 3.3 - 9.4% and 0.2 - 6.5%, respectively. The nature of the variation suggests that these differences are not merely due to environmental factors, but likely different genotypes (chemotypes).

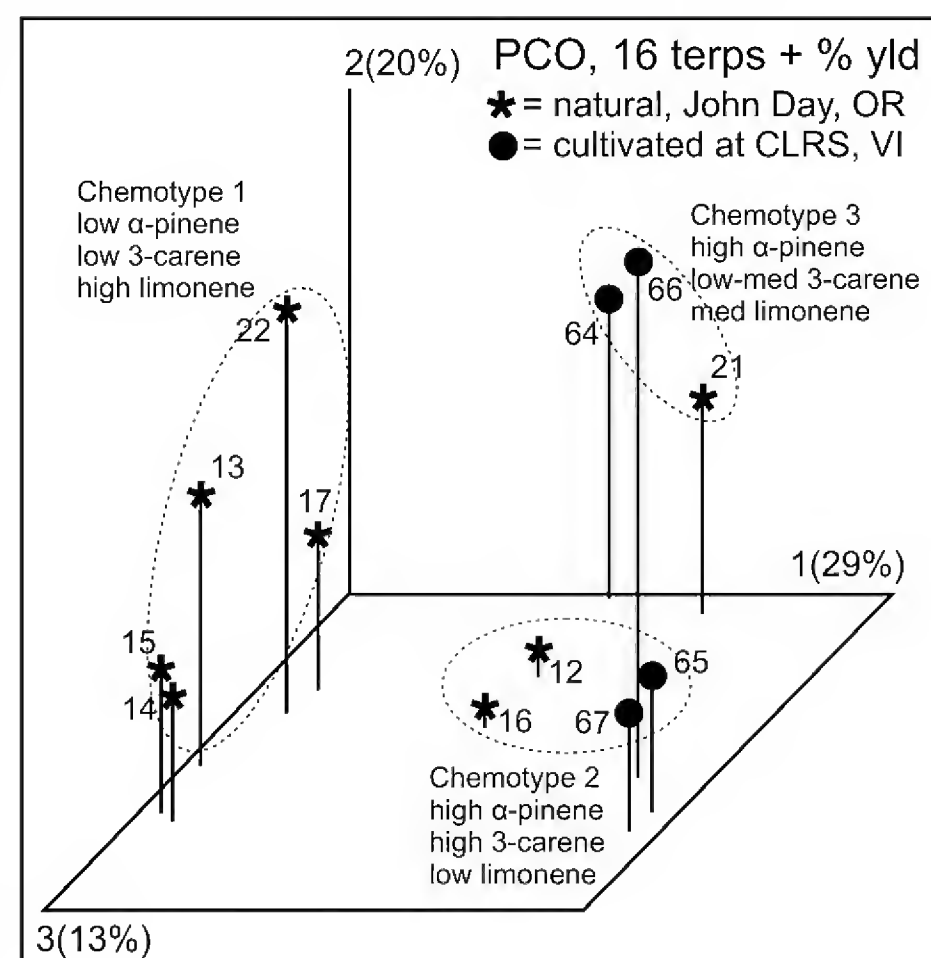


Figure 4. PCO of natural and cultivated samples (ex John Day, OR). 2 digit numbers are the last of collection number (eg., 21 = Adams 14121).

Correlation patterns among the 16 terpenoid concentrations and % oil yield (17 characters) were examined by PCA resulting in 3 eigenroots that accounted for 43.05, 22.14 and 17.51% of the variation among the 17 characters (82.75% of the total variance). Ordination of the 16 terpenoids plus % oil yield is shown in Figure 5. As seen in the Rennell Pass individuals (Fig. 3), LMNN and BPNN are negatively correlated with the monoterpenes. The other monoterpenes (APNN, 3CRN, BPNN, AFNC, MYRC, TRPN) are highly correlated and cluster (Fig. 5, left). Oil yield (% yld) has only low correlations (0.55 ATAC, -0.55 NZKL, 0.53 LMNN) as found in Case I (Fig. 3).

In summary, as in Case I, the environmental contribution to sample ordination seems less than the variation due to genetic differences as evidenced by the grouping of CLRS and natural trees. The large differences among the chemotypes in the concentrations of major terpenoids (Table 5) supports genetic control of terpene synthase activities of individuals, rather than merely environmental modification of enzyme pathways.

Case III. Oils from naturally grown compared to cultivated trees: Mt. Angeles (lower slope), ONP population.

Seeds were collected from trees on Mt. Angeles, ONP, germinated and planted at the JR site in 1994, thus producing mature trees for sampling (Nov. 2012). In addition, samples were collected from trees growing naturally on the lower slopes of Mt. Angeles for terpene analysis. ANOVA between Mt. Angeles natural and cultivated samples revealed (Table 6, % total oil basis) terpinolene and nezukol were highly significantly different, and trans-totarol was significantly different. Percent oil yield, although a little smaller in the natural population, was not significant. The differences between the major compounds were usually not significant, except for nezukol (5.32% natural, 17.2% cultivated, Table 6.).

The similarity matrix among the samples was factored and gave five eigenroots before they began to asymptote. The five eigenroots accounted for 34.1, 15.7, 12.7, 9.9, and 6.9% of the variation among the 11 samples (79.9% of total). In the PCO of the similarity matrix, axis 1 separates the samples into two chemotypes: 1. low limonene/ β - phellandrene, and high δ -3-carene and α -pinene and 2. high limonene/ β - phellandrene, medium δ -3-carene and medium or low α -pinene (Fig. 6). Chemotype 2 can be further subdivided by having medium amounts of α -pinene compared to low α -pinene (2a, Fig. 6).

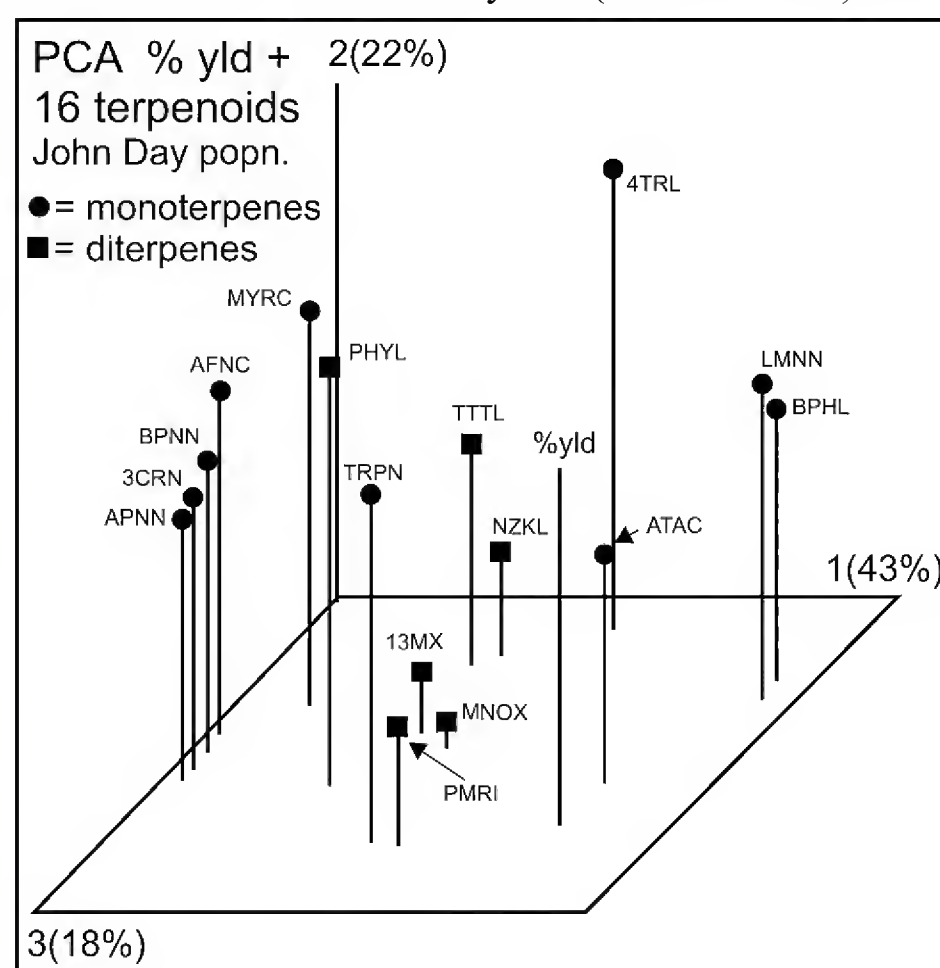


Figure 5. PCA showing the correlation patterns among terpenes and oil yield in the John Day popn.

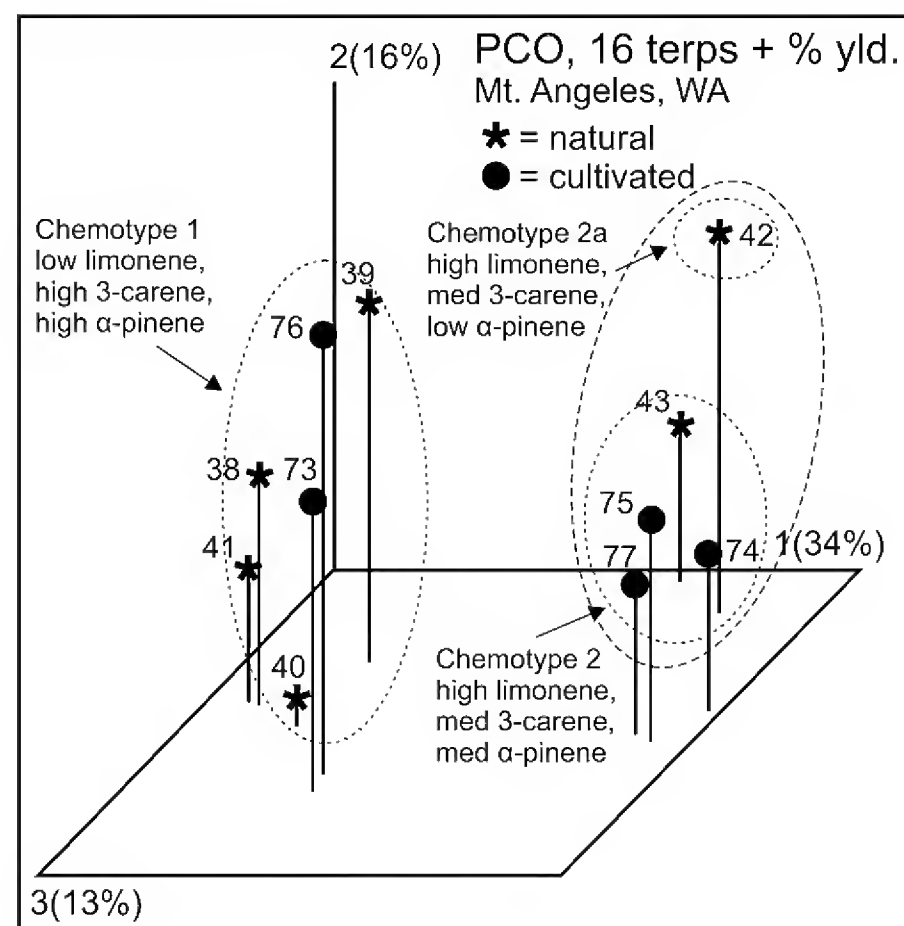


Fig. 6. PCO. Mt. Angeles, natural vs cultivated.

The two major chemotypes are interspersed in both the cultivated and natural samples (Fig. 6). The chemotypes seem to be little affected by the different environments at JR or Mt. Angeles, suggesting that genetics is the major factor in the maintenance of the chemotypes.

Table 7 shows the variation among the 3 chemotypes in representative samples of both cultivated and natural origins. Although chemotype 1 is uniformly high in α -pinene and δ -3-carene, with low limonene and β -phellandrene, nezukol varies from 3.4 to 17.7%. This same polymorphism is seen in chemotype 2, where nezukol ranges from 3.9 to 17.3% (Table 7).

The patterns of correlation among the 16 terpenoids and % oil yield (17 characters) were examined by PCA, which gave four eigenroots accounting for 47.3, 16.1, 12.2 and 10.3% of the variation among the 17 characters (85.9% of the variance). Ordination of the terpenoids plus % yield is shown in Figure 7. The pattern is similar to that found for the John Day data (Fig. 5), except the diterpene group (MNOX, 13MX, PMRI) is more closely associated with LMNN/BPHL. Once again, % oil yield is not strongly correlated (0.58 - LMNN and 0.58 - BPHL).

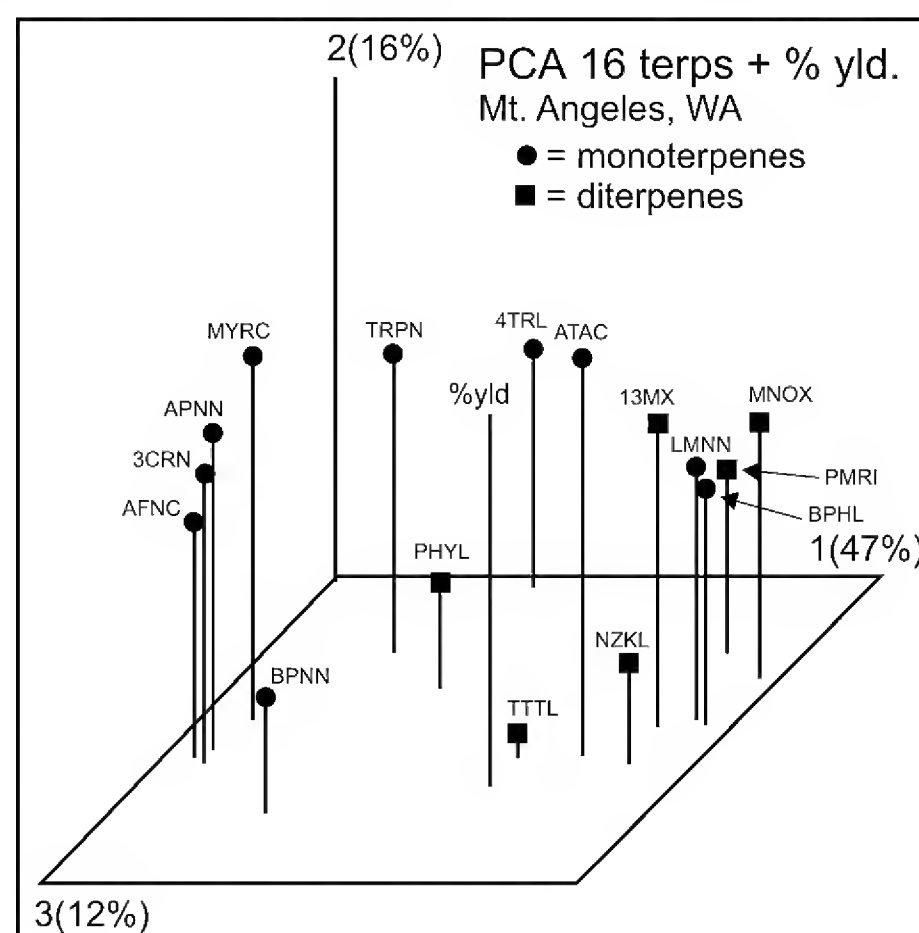


Figure 7. PCA, 16 terpenoids plus % yield, Mt. Angeles, WA.

The major pattern found in PCO was the interspersion of samples from cultivated and natural trees in the two major chemotypes (Fig. 6). This suggests that the major factor in determining the observed ordination is genetic differences.

Case IV. Oils from naturally grown compared to cultivated trees: Mitkof Island, AK population.

Cuttings were collected from trees on Mitkof Island, AK and subsequently, rooted cuttings were planted at the CLRS site in 1988 and leaf samples were taken (Nov. 2012) from 25 year old trees. In addition, samples were collected from trees growing naturally on Mitkof Island, AK (as near to the original collection site as possible) for terpene analysis.

ANOVA between natural samples from Mitkof Island and cultivated Mitkof Island trees (Table 8) revealed no significant differences in either the % oil yield or the concentrations of any of the 16 terpenoids.

PCO of the similarity matrix among the samples and ordination shows two chemotypes (Fig. 8, chemotypes 1 and 2). Chemotype 1 contains two cultivated trees (13713, 13714) and a natural tree (14153) whose oils have large amounts of α -pinene (24.9 - 31.0%), low amounts of limonene and β -phellandrene (2.5 - 5.5%) and moderate amounts of nezukol (7.1 - 11.3%). A fourth sample (natural, 14152) is similar, but differs in having lower α -pinene and a large amount of phyllocladanol (14.9%, Table 9, Chemotype 1a). In fact, this was the only sample (in either cultivated or natural groups) that had phyllocladanol (14.9%).

Chemotype 2 in PCO (Fig. 8) is composed of samples from 3 cultivated trees (13710, 13711, 13712) and 3 from natural trees (14149, 14150, 14151). The compositions of four of these samples are given in Table 9 (in boldface). All of these samples have low amounts of α -pinene (7.7 - 13.8%), large amounts of limonene/ β -phellandrene (14.0 - 18.9%) and moderate amounts of nezukol (5.5 - 10.8%). These data make it quite clear that there are 2 (and perhaps 3) chemotypes in the Mitkof Island population.

The fact that five of the samples came from cultivated trees on Vancouver Island and five came from a natural population on Mitkof Island did not appear to affect chemotypes compositions. In short, these chemotypes appear to be under strong genetic control of their terpene synthases.

To examine the pattern of correlation among the terpenes, PCA was performed and factoring the correlation matrix resulted in eigenroots that accounted for 46.8, 26.5, 10.2, 6.7 and 5.1% of the variation (total 95.3%). Ordination of the terpenoids (Fig. 9) show a quite different pattern than previously seen in the other related cases. The diterpenes are strongly correlated and form a tight group. Monoterpenes are in three groups: 1. α - and β - pinene, myrcene and δ -3-carene; 2. the balance of the monoterpene hydrocarbons plus α -terpinyl acetate and oil yield; and 3. terpin-4-ol (Fig. 9).

The highest correlations of oil yield are with α -terpinyl acetate (0.67) and terpinolene (0.61). The first axis removed 47% of the variation among the components. This may be due to the two chemotypes.

The Mitkof Island case presents the clearest evidence that differences in the oils of samples cultivated or from natural trees pale in comparison to the differences in individuals of either chemotype 1 or 2. There are surely environmentally induced differences between cultivated and natural tree oils from Mitkof Island, but they seem to be minor compared to the genotypic (chemotype) differences seen among these trees.

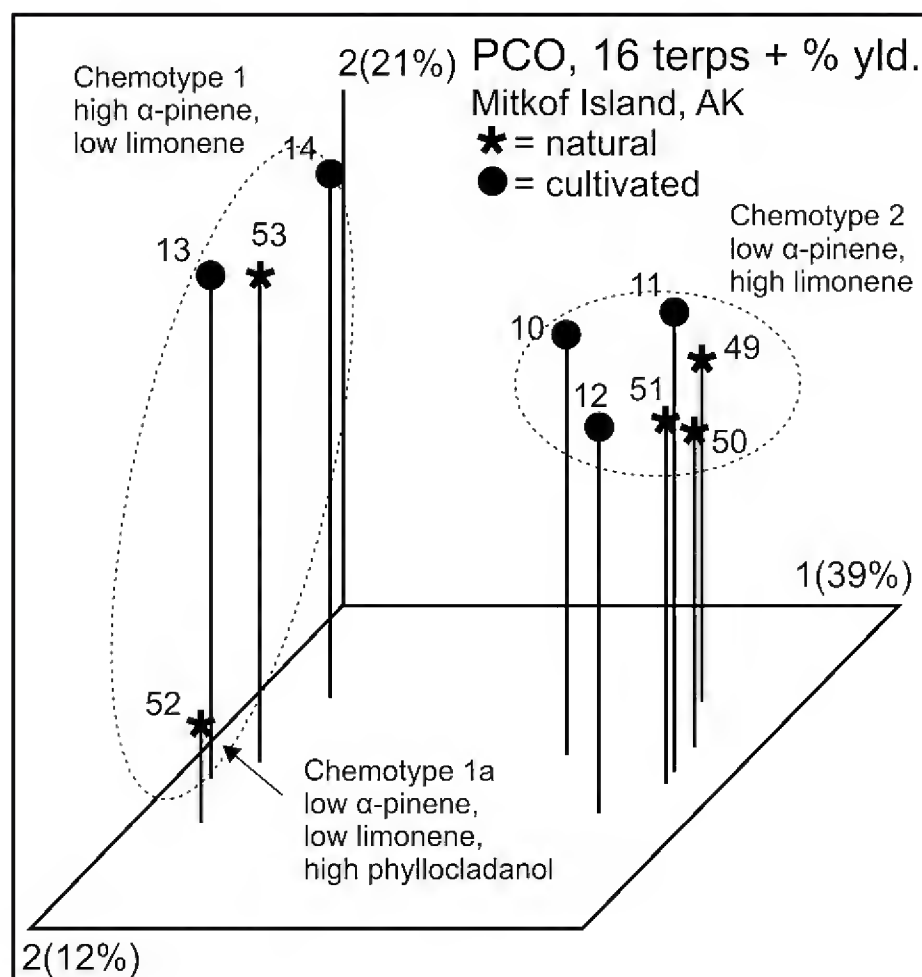


Figure 8. PCO of natural and cultivated Mitkof Island, AK samples.

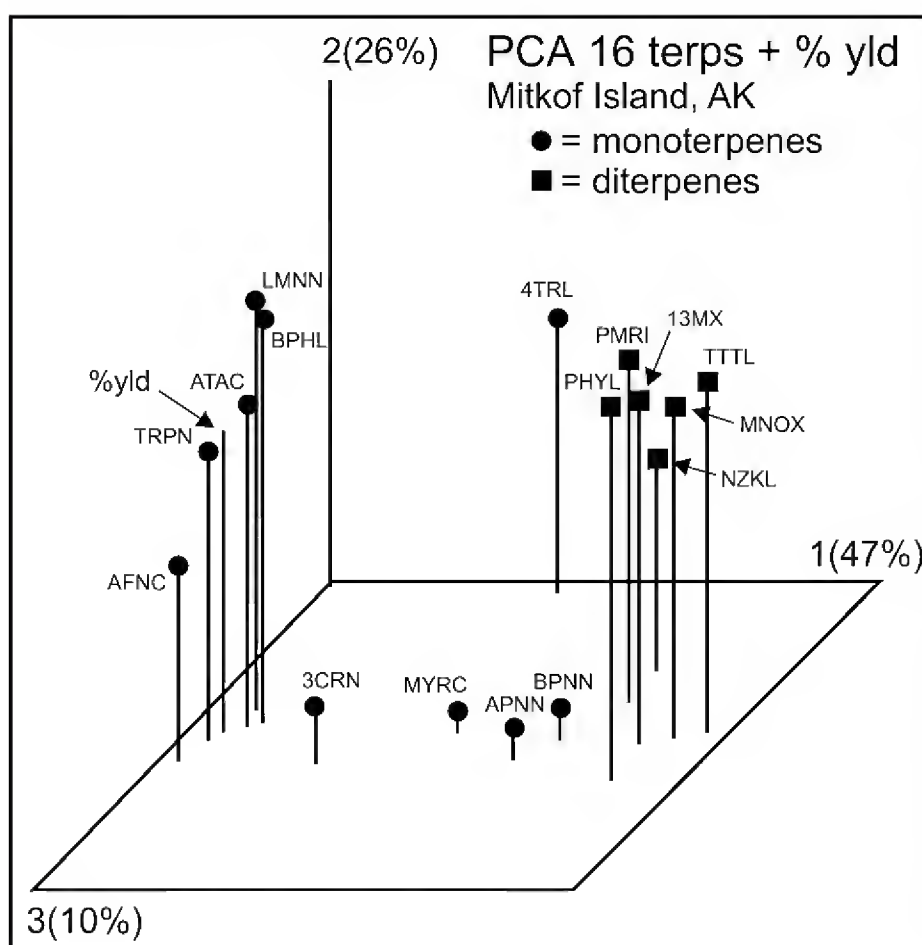


Figure 9. PCA, Mitkof Island, AK.

CONCLUSIONS

In general, the four cases examined showed similar results in that the samples from cultivated and natural trees were interspersed in PCO rather than clustering by source. This implies that genetics (chemotypes) plays a larger role than environmental factors for the cases examined. In all cases but the first, chemotypes were found and these exhibited large compositional differences from other oils in the same plot or natural population. These chemotypes (genotypes) persisted whether in cultivation or in the natural population. This is highly suggestive that genetics plays a strong role in the expression of terpene synthase genes in *C. nootkatensis*. Unexpectedly, the yields of oils from cultivated and natural trees were not significantly different in any of the four cases. However, it should be noted that all the samples were intentionally collected during the fall to minimize seasonal variation. So seasonal variation should not be dismissed as insignificant. The use of data, from either or both the CLRS, JR sites and/or from natural populations, appear to be justified by our studies.

ACKNOWLEDGEMENTS

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Table 1. ANOVA of % oil yields and leaf terpenoid concentrations (as % total oil) of *C. nootkatensis* from Rennell Pass grown at CLRS and JR plantations. ns = not significant at $P \leq 0.05$. * = significant ($P \leq 0.05$); ** = highly significant ($P \leq 0.01$). The 3 components that vary significantly between CLRS and JR are in boldface. Oil yields 2h corrected to 48h yield by correction factor of 4.34. Codes are used in the PCA figures throughout this paper.

KI	code	component	CLRS	JR	F ratio	P, signif.
	%yld	% oil yield (48h)	5.16	4.64	0.54	.508 ns
932	APNN	α -pinene	21.6	17.2	1.03	.347 ns
945	AFNC	α -fenchene	0.7	0.9	0.11	.751 ns
974	BPNN	β -pinene	2.5	2.0	1.21	.308 ns
988	MYRC	myrcene	3.2	2.2	9.74	.016 *
1008	3CRN	δ -3-carene	22.8	19.1	4.00	.084 ns
1024	LMNN	limonene	11.4	14.3	0.24	.642 ns
1025	BPHL	β -phellandrene	11.4	14.4	0.23	.647 ns
1086	TRPN	terpinolene	4.2	3.0	13.21	.008 **
1174	4TRL	terpinen-4-ol	0.8	0.3	3.16	.117 ns
1346	ATAC	α-terpinyl acetate	1.1	0.6	5.92	.044 *
1958	PMRI	pimaradiene<iso->	0.3	0.5	0.90	.624 ns
1987	MNOX	manoyl oxide	1.3	1.6	0.22	.881 ns
2009	13MX	13-epi-manool oxide	0.4	0.3	1.00	.648 ns
2132	NZKL	nezukol	7.6	12.9	4.09	.081 ns
2209	PHYL	phyllocladanol	0.3	1.6	1.84	.216 ns
2314	TTTL	trans-totarol	0.9	0.5	0.30	.605 ns

Table 2. ANOVA of oil yields and terpenoid concentrations as mg/ g DW of *C. nootkatensis* from Rennell South Pass grown at CLRS and JR plantations. ns = not significant at $P \leq 0.05$. * = significant ($P \leq 0.05$); ** = highly significant ($P \leq 0.01$). The 3 components that vary significantly between CLRS and JR are in boldface.

KI	component	CLRS	JR	F ratio	P, signif.
	oil yield (mg/g, 48h)	51.6	46.4	0.54	.508 ns
932	α -pinene	2.40	1.80	2.54	.153 ns
945	α -fenchene	0.10	0.09	0.44	.532 ns
974	β -pinene	0.27	0.20	2.56	.151 ns
988	myrcene	0.37	0.24	18.82	.004 **
1008	δ -3-carene	0.27	0.20	4.34	.074 ns
1024	limonene	1.53	1.49	0.01	.951 ns
1025	β -phellandrene	1.53	1.49	0.01	.944 ns
1086	terpinolene	0.56	0.31	6.78	.034 *
1174	terpinen-4-ol	0.09	0.05	2.28	.173 ns
1346	α-terpinyl acetate	0.14	0.06	5.53	.048 *
1958	pimaradiene<iso->	0.03	0.04	0.32	.592 ns
1987	manoyl oxide	0.17	0.15	0.15	.708 ns
2009	13-epi-manool oxide	0.05	0.03	1.29	.293 ns
2132	nezukol	0.83	1.52	2.97	.126 ns
2209	phyllocladanol	0.02	0.26	1.69	.233 ns
2314	trans-totarol	0.10	0.07	0.712	.569 ns

Table 3. Variation among the leaf essential oils (as % total oil) of *C. nootkatensis* from Rennell Sound Pass grown at CLRS and JR plantations. Note: 06 = Adams 13706, 08 = Adams 13708, 09 = Adams 13709, 01 = Adams 13701, 04 = Adams 13704, 05 = Adams 13705, in Figs. 1 and 2. The 3 components that vary significantly between CLRS and JR are in boldface.

KI	component	JR 13706	JR 13708	JR 13709	CLRS 13701	CLRS 13704	CLRS 13705
	% oil yield (48h)	4.81	4.64	3.82	5.90	3.12	6.25
932	α -pinene	17.6	21.8	14.0	18.1	35.4	9.3
945	α -fenchene	1.0	0.8	0.7	1.1	0.8	0.7
974	β -pinene	1.3	2.1	1.4	2.1	3.8	1.1
988	myrcene	2.3	2.1	1.9	2.7	4.3	2.8
1008	δ -3-carene	19.5	21.1	17.0	25.2	26.7	15.8
1024	limonene	18.5	7.5	15.4	11.9	4.7	24.6
1025	β -phellandrene	18.4	7.5	15.4	12.0	4.7	24.6
1086	terpinolene	3.8	2.0	3.3	4.4	4.1	4.3
1174	terpinen-4-ol	0.6	0.2	0.3	0.8	0.8	0.9
1346	α-terpinyl acetate	0.4	0.4	0.3	1.0	0.7	1.3
1958	pimaradiene<iso->	0.2	0.3	0.7	0.2	0.1	0.2
1987	manoyl oxide	0.8	1.9	1.6	1.6	0.9	0.9
2009	13-epi-manool oxide	0.2	0.5	0.3	0.5	0.3	0.3
2132	nezukol	5.7	23.0	18.7	8.5	3.6	3.6
2209	phyllocladanol	1.6	t	0.0	0.0	0.0	0.8
2314	trans-totarol	0.5	0.8	0.7	1.3	0.3	0.4

Table 4. ANOVA of % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Cedar Grove Botanical Area, CGBA, John Day, OR) and trees from cuttings (ex CGBA) cultivated at CLRS. ns = not significant at $P \leq 0.05$. * = significant ($P \leq 0.05$); ** = highly significant ($P \leq 0.01$). Components that vary significantly are in boldface.

KI	component	CGBA natural	CGBA CLRS	F ratio	P, signif.
	% yield (48h)	4.38	3.52	1.36	.270 ns
932	α-pinene	11.51	34.17	29.97	.005 **
945	α -fenchene	0.93	0.70	2.06	.179 ns
974	β-pinene	1.14	3.40	31.90	.004 **
988	myrcene	2.26	3.55	47.60	.001 **
1008	δ -3-carene	19.38	19.27	0.00	.981 ns
1024	limonene	18.99	7.25	8.01	.017 *
1025	β-phellandrene	18.99	7.25	8.01	.017 *
1086	terpinolene	3.04	2.60	1.34	.273 ns
1174	terpinen-4-ol	0.11	0.16	nt	nt
1346	α -terpinyl acetate	0.73	0.62	0.08	.776 ns
1958	pimaradiene<iso->	0.69	1.20	3.01	.111 ns
1987	manoyl oxide	3.30	2.25	2.27	.164 ns
2009	13-epi-manool oxide	0.99	0.68	4.09	.068 ns
2132	nezukol	7.74	6.93	0.13	.723 ns
2209	phyllocladanol	0.50	0.93	0.50	.504 ns
2314	trans-totarol	0.27	0.25	nt	.nt ns

Table 5. Variation in % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* for natural individuals from CGBA (John Day, OR) and cultivated individuals grown from cuttings (ex CGBA) at CLRS, Vancouver Island, BC. Notes: In Fig. 4: 13 = *Adams 14113*, 14 = *Adams 14114*, 16 = *Adams 14116*, 65 = *Adams 13665*, 67 = *Adams 13667*, 64 = *Adams 13664*, 66 = *Adams 13666*, 12 = *Adams 14121*. Chemotypes 1, 2 and 3 are shown in normal, italics and boldface.

KI	component	chemotype 1		chemotype 2			chemotype 3		
		CGBA 14113	CGBA 14114	CGBA 14116	CLRS 13665	CLRS 13667	CLRS 13664	CLRS 13666	CGBA 14121
	% yield (48h)	6.55	5.99	4.43	3.39	3.47	4.38	2.91	3.82
932	α -pinene	7.8	7.1	15.0	34.8	32.4	24.6	44.9	22.2
945	α -fenchene	0.7	0.8	1.1	0.9	0.9	0.6	0.4	1.4
974	β -pinene	0.8	0.8	1.8	3.6	3.8	2.1	4.1	1.9
988	myrcene	2.2	2.1	2.4	3.7	3.4	3.1	4.0	2.4
1008	δ -3-carene	11.7	15.6	25.1	23.7	26.1	16.3	11.0	33.3
1024	limonene	26.4	22.8	11.7	3.0	3.3	14.2	8.5	7.2
1025	β -phellandrene	26.4	22.8	11.7	3.0	3.3	14.2	8.5	7.2
1086	terpinolene	3.6	2.7	4.0	2.6	3.2	2.6	2.0	3.4
1174	terpinen-4-ol	0.1	0.1	0.1	0.0	0.0	0.6	0.1	0.0
1346	α -terpinyl acetate	0.8	1.7	0.6	0.4	0.3	0.5	1.3	0.0
1958	pimaradiene<iso->	0.5	0.9	1.1	1.5	1.2	0.2	1.9	0.7
1987	manoyl oxide	2.4	3.9	4.4	2.7	3.5	2.4	0.4	2.8
2009	13-epi-manool oxide	0.7	1.1	1.1	0.4	0.8	9.9	0.6	1.0
2132	nezukol	3.3	9.4	9.7	11.2	9.8	6.5	0.2	5.3
2209	phyllocladanol	0.0	0.0	0.0	0.0	0.0	1.5	2.2	2.1
2314	trans-totarol	0.1	0.2	0.4	0.2	0.4	0.2	0.2	0.2

Table 6. ANOVA of oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mt. Angeles, ONP) and trees (cuttings from Mt. Angeles) cultivated at JR. ns = not significant at P=0.05. * = significant (P≤ 0.05); ** ≤ highly significant (P ≤ 0.01). Components that vary significantly are in boldface.

KI	component	Mt. Angeles natural	Mt. Angeles CLRS	F ratio	P, signif.
	% yield (48h)	4.69	4.77	0.10	.919 ns
932	α-pinene	21.72	14.70	1.73	.219 ns
945	α-fenchene	1.05	0.88	2.34	.158 ns
974	β-pinene	2.22	3.10	0.70	.571 ns
988	myrcene	2.67	2.22	4.70	.056 ns
1008	δ-3-carene	27.10	19.58	4.00	.074 ns
1024	limonene	9.15	11.92	0.46	.521 ns
1025	β-phellandrene	9.13	11.90	0.46	.522 ns
1086	terpinolene	3.67	2.96	17.38	.003 **
1174	terpinen-4-ol	0.78	0.48	1.51	.249 ns
1346	α-terpinyl acetate	0.38	0.86	1.52	.248 ns
1958	pimaradiene<iso->	0.32	0.48	2.56	.142 ns
1987	manoyl oxide	1.20	1.58	1.39	.268 ns
2009	13-epi-manool oxide	0.32	0.34	0.08	.775 ns
2132	nezukol	5.32	17.20	50.43	.0002 **
2209	phyllocladanol	1.62	0.68	1.23	.230 ns
2314	trans-totarol	0.35	0.78	6.11	.034 *

Table 7. Variation in oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* for natural individuals at Mt. Angels (Mt Ang), ONP and cultivated individuals grown from seed at JR, Vancouver Island, BC. Notes: In Fig. 6: 38 = *Adams 14138*, 40 = *Adams 14140*, 42 = *Adams 14142*, 73 = *Adams 13673*, 76 = *Adams 13676*, 43 = *Adams 14143*, 74 = *Adams 13674*, 77 = *Adams 13677*, 42 = *Adams 14142*. Chemotypes 1, 2 and 2a are in normal, boldface and boldface/italics fonts.

KI	component	chemotype 1				chemotype 2			ch. 2a
		Mt Ang 14138	Mt Ang 14140	CLRS 13673	CLRS 13676	Mt Ang 14143	CLRS 13674	CLRS 13677	<i>Mt Ang 14142</i>
	% yield (48h)	4.25	5.21	3.82	4.90	7.25	5.47	4.82	4.04
932	α-pinene	28.8	28.0	20.8	17.0	13.4	10.6	10.5	5.3
945	α-fenchene	1.1	1.3	1.1	1.1	1.0	0.7	0.7	0.8
974	β-pinene	2.7	2.8	2.9	7.2	1.3	1.2	1.9	0.8
988	myrcene	2.9	2.9	2.4	2.1	2.5	2.0	2.2	1.9
1008	δ-3-carene	32.5	36.6	25.7	25.8	22.1	16.3	15.3	18.3
1024	limonene	3.5	3.3	5.3	8.3	19.9	16.8	13.8	18.7
1025	β-phellandrene	3.5	3.3	5.3	8.3	19.9	16.8	13.8	18.7
1086	terpinolene	3.7	4.0	3.3	3.1	3.6	2.7	3.1	3.8
1174	terpinen-4-ol	1.2	0.4	0.6	0.1	0.5	0.2	1.3	0.7
1346	α-terpinyl acetate	0.0	1.3	0.2	0.0	0.8	1.6	1.6	0.0
1958	pimaradiene<iso->	0.4	0.2	0.4	0.3	0.3	0.5	0.8	0.5
1987	manoyl oxide	0.6	1.1	1.2	0.9	1.4	1.7	2.1	2.1
2009	13-epi-manool oxide	0.1	0.3	0.3	0.2	0.5	0.4	0.3	0.4
2132	nezukol	3.4	3.9	17.7	15.3	3.9	17.3	18.2	11.5
2209	phyllocladanol	1.4	0.0	0.1	3.3	1.1	0.0	0.0	2.8
2314	trans-totarol	0.3	0.1	1.3	0.9	0.3	0.4	0.5	0.7

Table 8. ANOVA of % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mitkof Island, AK) and trees (seeds from Mitkof Isl.) cultivated at CLRS. ns = not significant at P=0.05.

KI	component	Mitkof Island natural	Mitkof Island CLRS	F ratio	P, signif.
	% yield (48h)	3.08	2.69	1.14	.319 ns
932	α -pinene	13.34	18.38	0.69	.567 ns
945	α -fenchene	0.76	0.82	0.54	.501 ns
974	β -pinene	1.42	2.26	1.31	.286 ns
988	myrcene	2.48	2.96	2.30	.165 ns
1008	δ -3-carene	19.08	21.42	1.12	.323 ns
1024	limonene	10.32	12.74	0.25	.630 ns
1025	β -phellandrene	10.26	12.64	0.25	.635 ns
1086	terpinolene	3.94	4.06	0.41	.544 ns
1174	terpinen-4-ol	1.08	1.18	0.13	.726 ns
1346	α -terpinyl acetate	1.54	0.72	3.23	.108 ns
1958	pimaradiene<iso->	0.28	0.74	3.07	.115 ns
1987	manoyl oxide	3.14	3.02	0.02	.892 ns
2009	13-epi-manool oxide	1.14	0.90	0.37	.566 ns
2132	nezukol	11.02	7.62	3.22	.108 ns
2209	phyllocladanol	2.98	0.00	1.00	.652 ns
2314	trans-totarol	0.50	0.36	0.40	.552 ns

Table 9. Variation in oil yields and terpenoids (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mitkof Island, AK) and trees (seeds from Mitkof Isl.) cultivated at CLRS. No significant differences were found in ANOVA. Note: In Fig. 8: 53 = *Adams 14153*, 13 = *Adams 13713*, 14 = *Adams 13714*, 52 = *Adams 14152*, 49 = *Adams 14149*, 50 = *Adams 14150*, 10 = *Adams 13710*, 11 = *Adams 13711*. Chemotypes 1, 1a and 2 (see Fig. 8) are in normal, italics and boldface fonts.

KI	component	chemotype 1			ch. 1a	chemotype 2			
		Mitkof 14153	CLRS 13713	CLRS 13714	<i>Mitkof 14152</i>	Mitkof 14149	Mitkof 14150	CLRS 13710	CLRS 13711
	% yield (48h)	2.53	2.47	2.39	2.26*	3.12	3.43	3.43	2.47
932	α -pinene	31.0	31.0	24.9	9.1	10.7	7.7	13.8	10.4
945	α -fenchene	0.7	0.9	0.7	0.5	0.8	0.9	0.8	0.8
974	β -pinene	3.0	3.8	3.7	1.3	1.1	0.8	1.3	1.5
988	myrcene	3.2	3.4	3.6	1.9	2.4	2.4	2.9	2.4
1008	δ -3-carene	19.0	28.7	20.1	14.7	20.5	20.1	17.6	20.0
1024	limonene	3.6	2.6	5.5	2.4	14.0	15.8	18.9	15.2
1025	β -phellandrene	3.6	2.5	5.4	2.4	14.0	15.7	18.8	15.1
1086	terpinolene	2.9	4.1	3.8	3.2	4.3	4.4	4.1	3.9
1174	terpinen-4-ol	0.9	0.7	1.6	0.8	1.7	1.0	1.3	1.7
1346	α -terpinyl acetate	0.6	0.5	0.4	0.5	2.2	2.1	0.3	1.0
1958	pimaradiene <iso->	0.4	0.6	0.7	0.5	0.3	0.1	0.4	1.7
1987	manoyl oxide	2.9	3.2	3.3	6.6	2.0	2.2	3.2	3.0
2009	13-epi-manool oxide	0.9	1.0	0.9	2.7	0.7	0.7	1.0	0.7
2132	nezukol	11.3	7.1	11.3	15.3	9.2	9.9	5.5	10.8
2209	phyllocladanol	0.0	0.0	0.0	14.9	0.0	0.0	0.0	0.0
2314	trans-totarol	0.5	0.3	0.6	1.3	0.3	0.2	0.2	0.5

*14152 had copious amounts of white, crystalline distillate (diterpenes) that precipitated in the condenser. Thus, the oil yield for 14152 is under estimated.

Geographic variation in the leaf essential oil of *Juniperus turbinata* from throughout its range in the Mediterranean

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ABSTRACT

The volatile leaf oils from sixteen populations of *Juniperus turbinata* (= *J. phoenicea* subsp. *turbinata*) were analyzed. The variation in the major components, α -pinene (17.7 - 67.9%) and β -phellandrene (0.5 - 31.5%), was extremely large. Overall, the oils are quite variable as one might expect from the great variation in habitats ranging from coastal (8 - 15 m) to high mountains (Algeria, 1451 m, Morocco, 940 m) and from desert (Sinai) to Mediterranean. Considerable geographical variation was found in the oils dividing the populations into five groups: Portugal - Spain; Mediterranean Basin - Madeira; Canary Islands; High Atlas mountains and Sinai. There appears to be more of a mosaic than continuous geographical pattern among the populations. The utilization of oil from 12 year old herbarium specimens was examined by comparing with fresh leaves from Crotone, Italy. It appears likely that both oxidation and free radical reactions have occurred in the 12 year old herbarium leaves. In this study, it was not possible to utilize oils from herbarium specimens. Published on-line www.phytologia.org *Phytologia* 96(3): 149-158 (July 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus turbinata*, *J. phoenicea*, var. *turbinata*, *Cupressaceae*, leaf essential oils geographic variation.

The genus *Juniperus* is comprised of approx. 75 species in 3 sections (Adams, 2014) with serrate (denticulate) leaf-margined species found in both the eastern hemisphere (1 species) and western hemisphere (21 species). *Juniperus phoenicea* and *J. turbinata* are the only serrate-leaf juniper in the eastern hemisphere. They have been treated as *J. p.* var. *phoenicea* and var. *turbinata* (Adams, 2011) or as subsp. *turbinata* (Farjon, 2005). However, Adams and Schwarzbach (2013) have recently shown that *J. phoenicea* is not part of a clade of serrate-leaf junipers occurring in the western hemisphere, leading them to denote *J. phoenicea* as a 'pseudoserrate' juniper. In addition, they found *J. p.* var. *phoenicea* and var. *turbinata* to be as different in their DNA sequences as several other recognized species of *Juniperus*, lending support for the recognition of *J. turbinata* Guss. as proposed by Lebreton and Perez de Paz (2001) based largely on the concentration of prodelphinidin, a polymeric tannin. The prodelphinidin data suggested that *J. phoenicea* var. *phoenicea* was confined to the Iberian Peninsula and south France, with var. *turbinata* being widespread throughout the Mediterranean. Farjon (2005) considered subsp. *phoenicea* to be widespread in the Mediterranean and subsp. *turbinata* to be confined to littoral maritime habitats (sand and rocks). Adams (2011) followed the distributions of Farjon (2005), except for the Canary Islands and Madeira, which, based on DNA sequence data, have been shown (Adams et al. 2010) to be *J. turbinata* not *J. phoenicea* var. (subsp.) *phoenicea*.

The most comprehensive DNA sequence study to date (Adams et al. 2013) sampled 2 populations of *J. phoenicea* var. *phoenicea* and 19 populations of *J. phoenicea* var. *turbinata*. They found *J. phoenicea* var. *phoenicea* in a separate clade from *J. p.* var. *turbinata* (Fig. 1), with *J. phoenicea* var. *phoenicea* only found in the two Spain populations. *Juniperus turbinata* from the Canary Islands and Madeira, through the Mediterranean and eastward to the Sinai were all in a distinct clade (Fig. 1). It is this classification that was used for the basis of selecting *J. turbinata* populations for this study.

The volatile leaf oils of *J. phoenicea*, *sensu stricto*, have been recently examined (Adams, Altarejos and Arista, 2014) and the literature on the leaf oils of *J. phoenicea* and *J. p.* var. *turbinata* (*J. turbinata*) has been reviewed therein.

The purpose of this paper is to present new data on geographic variation in the volatile leaf oils of *J. turbinata* from throughout its range (Fig. 2).

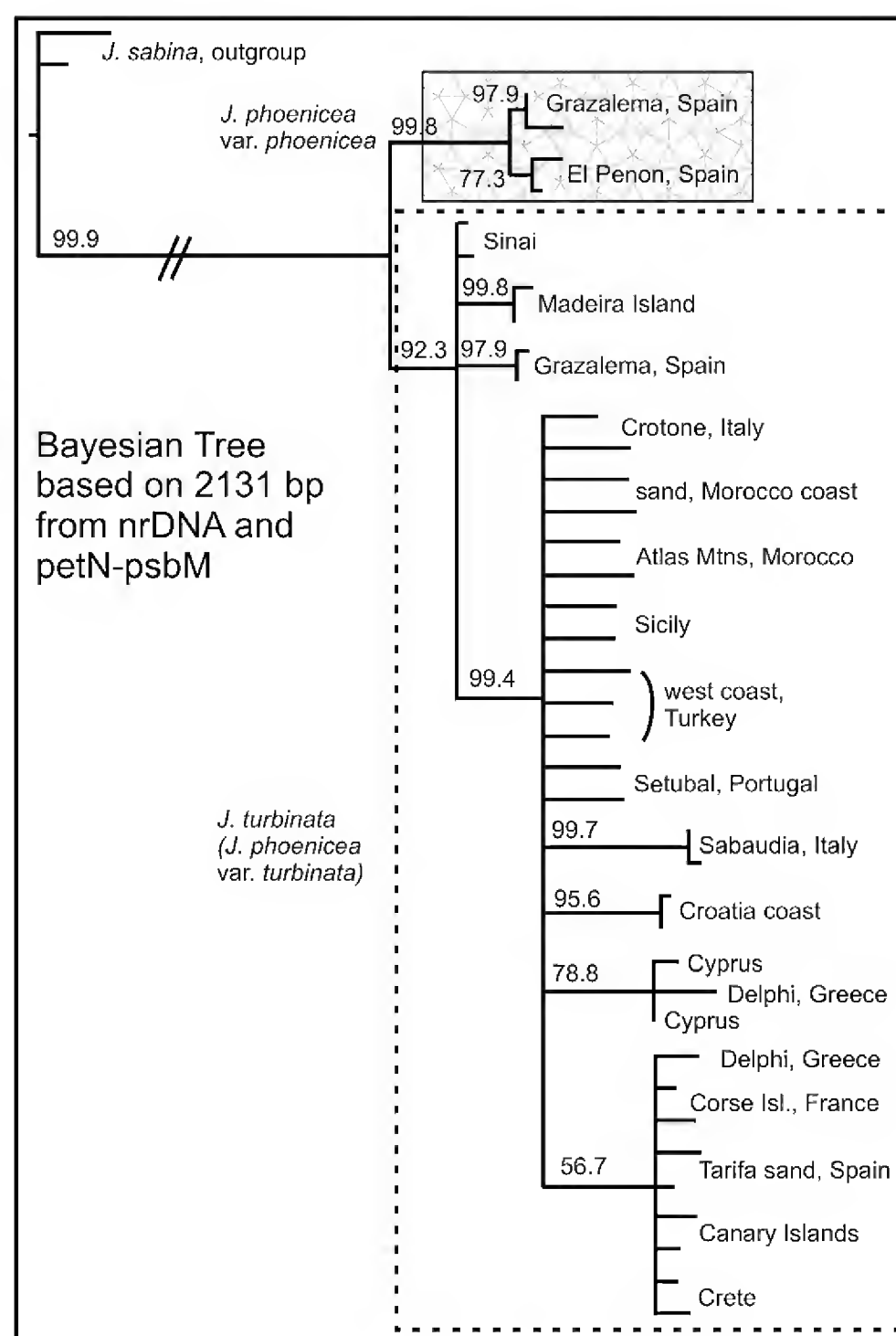


Figure 1. Bayesian tree showing *J. phoenicea* and *J. turbinata* (*J. p.* var. *phoenicea*) in well-supported clades. From Adams et al. 2013

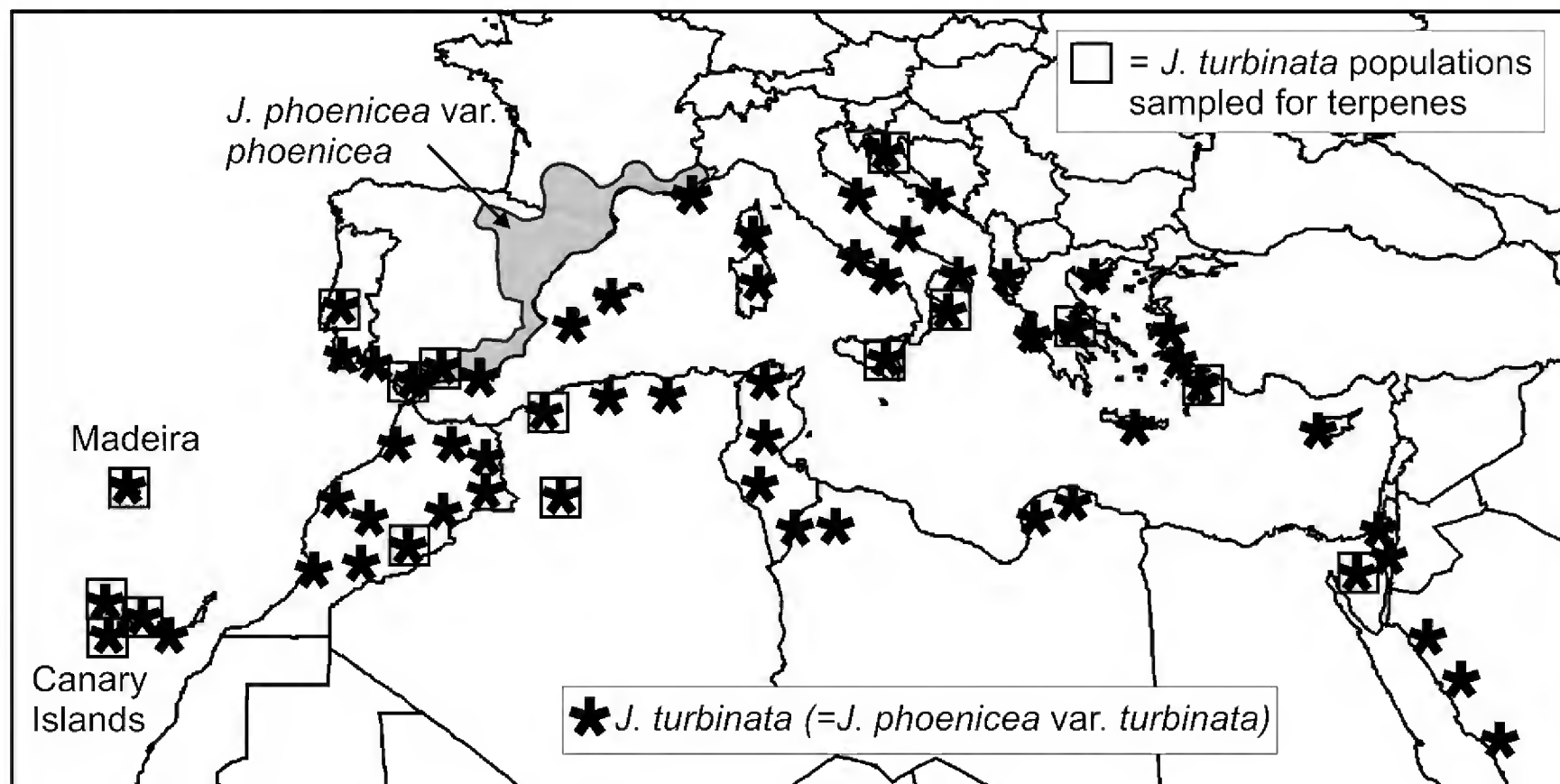


Figure 2. Distribution of *J. phoenicea* (adapted from Lebreton and Perez de Paz (2001) and Adams et al. (2010). Squares show populations *J. turbinata* sampled in the present study.

MATERIALS AND METHODS

Specimens used in this study, *J. turbinata*:

Algeria, inland Mountains (continental), 32° 41.332' N; 00° 29.003' E, 1451 m, *H. Houari*, 1-6, *Adams* 13984-13989, 23 April 2013 (fresh),

Algeria, coastal (littoral), 35° 47.374' N, 00° 29.074' E, 77 m, *H. Houari*, 7-12, *Adams* 13990-13995, (fresh),

Canary Islands, La Gomera, 28° 11.358' N; 17° 12.320' W, 370 m, *Adams* 11528-11530, (fresh),

Canary Islands, Tenerife, 0.5km S. of Tejina de Isora on rt. 822, 29° 10' 48" N, 16° 45' 53" W, ca. elev. 520m, *Adams* 8147-8149, (fresh),

Canary Islands, La Palma, 28° 44.250' N; 017° 44.198' W, 283 m, *Adams* 11514-11517, (fresh),

Croatia, Ugljan Island, 44° 05' 0.27" N, 15° 09' 39.29" E, elev. 20-32 m, *Zlato Liber* 1-5, Baylor specs. *Adams* 13589-13593, (fresh),

Greece, north of Nea Epidavos, 37° 41.091' N; 23° 07.686' E, 76 m, *Adams* 9439-9441, (fresh),

Italy, Crotone (south coast)

Adam Boratynski IT-2(1-5), Baylor specs. *Adams* 13341-13345, 38° 53' 36" N, 17° 05' 42" E, elev. 10 m, 9 Dec 2001 (12 yr old herbarium specimens),

Pietro Minissale & Saverio Sciandrello 1-6, *Adams* 14105-14110, Capo Rizzuto, near sea side. 38° 53' 36" N, 17° 05' 42" E, elev. 10 m, fresh, 7 Sep 2013, (same population as *Boratynski* above),

Madeira Island, Portugal, elev. ca. 20m, *Adams* 11502-11504, (fresh),

Morocco, rd to Oukaimeden, 31° 21.033' N, 07° 45.893' W, elev. 940m, *Adams* 9408-9410, (fresh),

Portugal, Setubal, Sa. da Arrabida Mountains, 12 km west of Setubal on N379-1, 225m. *Adams* 7074-7076, (fresh)

Sicily, near Piano Pirrera near Acate (Ragusa), 37° 01' 35.75" N; 14° 26' 07.86" E., 120 m, *Pietro*

Minissale & Saverio Sciandrello 1-5, Baylor specs. *Adams* 13778-13782, 18 Jan 2013, (fresh),

Sinai, 30°38'09"N, 33°26'53"E, elev. 700 m *Hagar Leschner* 1-5, Baylor specs. *Adams* 13495-13499, 15 Jun 2012 (fresh),

Spain, Sierra de Grazalema, 36° 48' 10.9"N, 5° 24' 21.2"W, elev. 829m, *M. Arista* 6-10, Baylor specs.

Adams 13818-13822 (fresh),

Spain, Tarifa sand dunes, elev. ca. 20m, *Adams* 7202-7204, (fresh),

Turkey, Mugla, main road between Marmaris - Datca, 36° 46' N; 27° 59' E, 308 m, *Tugrul Mataraci* 1-4,

Adams 13969-13972, (fresh),

Voucher specimens are deposited at BAYLU herbarium Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Additional steam distillation tests for up to 48 h revealed that the 2 h distillation removed about 23% (of the total oil for 48 h), providing a correction factor of 4.34 (x 2h = 48 h total). The yields appeared to asymptote at about 104% of the 48 h total oil.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (Gower, 1971; Adams, 1975). Principal Coordinate Ordination (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The compositions of the leaf volatile oils for several populations are shown in table 1. The variation in the major components, α -pinene (17.7 - 67.9%) and β -phellandrene (0.5 - 31.5%), is extremely large. Overall, the oils are highly variable as one might expect from the great variation in habitats ranging from coastal (8 - 15 m) to high mountains (Algeria, 1451 m, Morocco, 940 m) and from desert (Sinai) to Mediterranean.

To further examine geographic variation, PCO (Principal Coordinate Ordination) was performed on the oils from the 16 populations using 29 terpenoids (* in table 1). Six eigenroots accounted for 24.5, 14.1, 10.1, 9.0, 6.2, and 5.9% of the variation (69.8% of total variance) before the eigenroots began to asymptote. The low amount of variance accounted for in the first few eigenroots suggests the variance cannot be explained for only a few trends among the populations. Ordination of the 16 *J. turbinata* populations (Fig. 3) shows four major groupings:

1. Portugal - Spain (GS, Grazalema, Spain; TS, Tarifa sands, Spain; PO, Setubal, Portugal);
2. Mediterranean: Italy, Greece, Sicily, Algeria (coastal), Croatia, Turkey and Madeira;
3. Canary Islands: TC, Tenerife; PC, La Palma; GC, La Gomera;
4. High Atlas mountains: Algeria, Morocco;
5. Sinai.

It is interesting that Madeira oil seems more associated with the central Mediterranean than with nearby Canary Islands oils (Fig. 3). However, it should be noted that only 45% of the variance among populations was accounted for in the PCO ordination (Fig. 3), so relationships between individual populations is distorted by ordination in only three dimensions.

To further examine geographical trends, similarity measures were calculated (using 29 terpenoids) and populations were clustered using a minimum spanning network. The clustering was then contoured mapped to present a geographical representation of trends (Fig. 4). The most similar populations were Tenerife and La Palma, Canary Islands, at 0.86 similarity (Fig. 4), followed by Spain (GR, Grazalema, TS, Tarifa sands, 0.82) and Greece - Turkey (0.82). In deference to the PCO (Fig. 3), Croatia and Sicily were quite similar (0.81, Fig. 4). The Atlas Mountains populations (Morocco, Algeria Mtns.) clustered at 0.80. But the Algerian coastal population clustered with Madeira, Canary Islands, Croatia - Sicily and Greece - Turkey populations that comprised the central Mediterranean group as seen in the PCO (Fig. 3). The final group entering the cluster was the Atlas Mountains group (Morocco, Algeria Mtns.) that are the most differentiated, joining with all the other populations of *J. turbinata* at a similarity of 0.75 (Fig. 4).

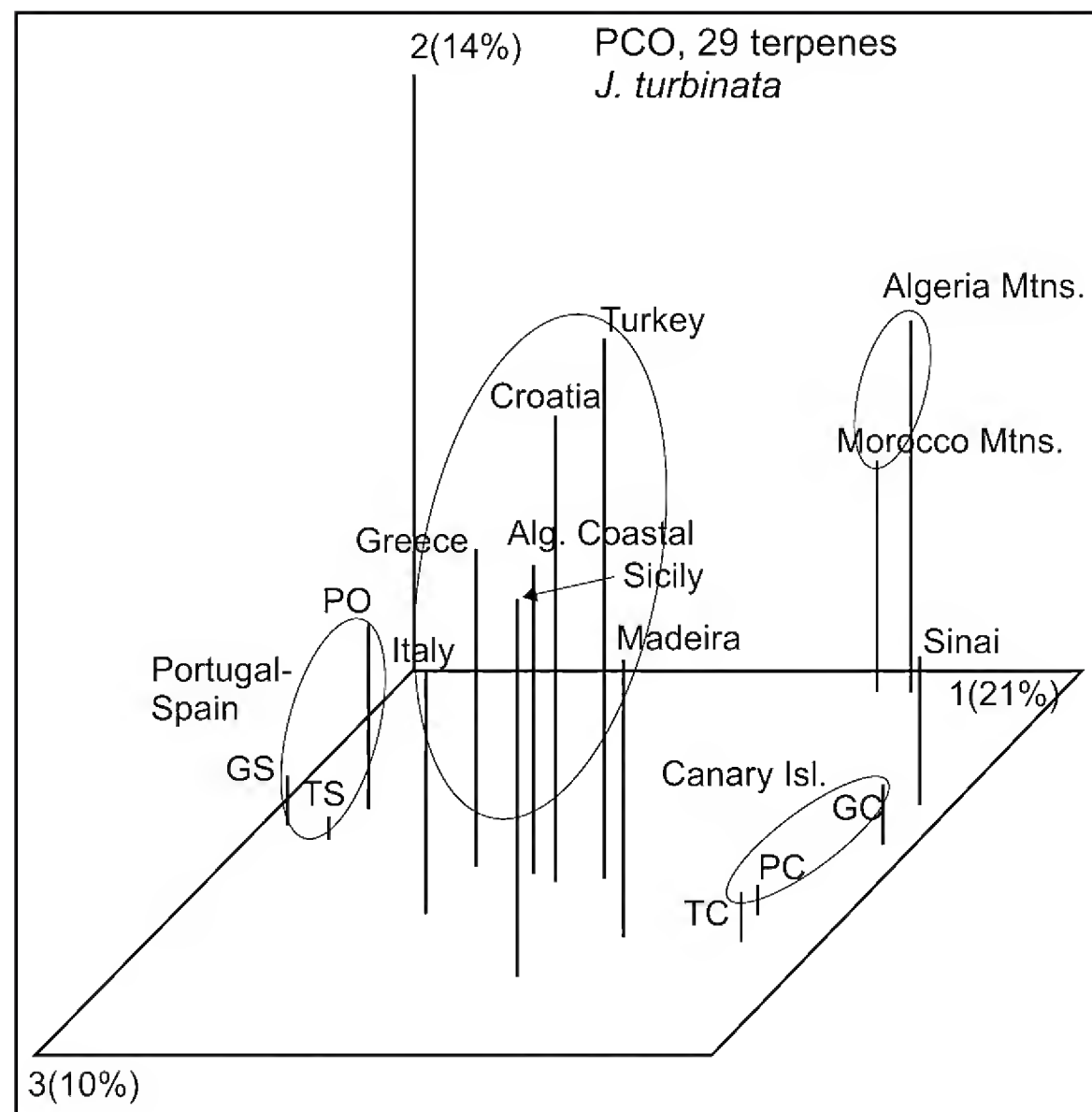


Figure 3. PCO of *J. turbinata* based on 29 terpenes.

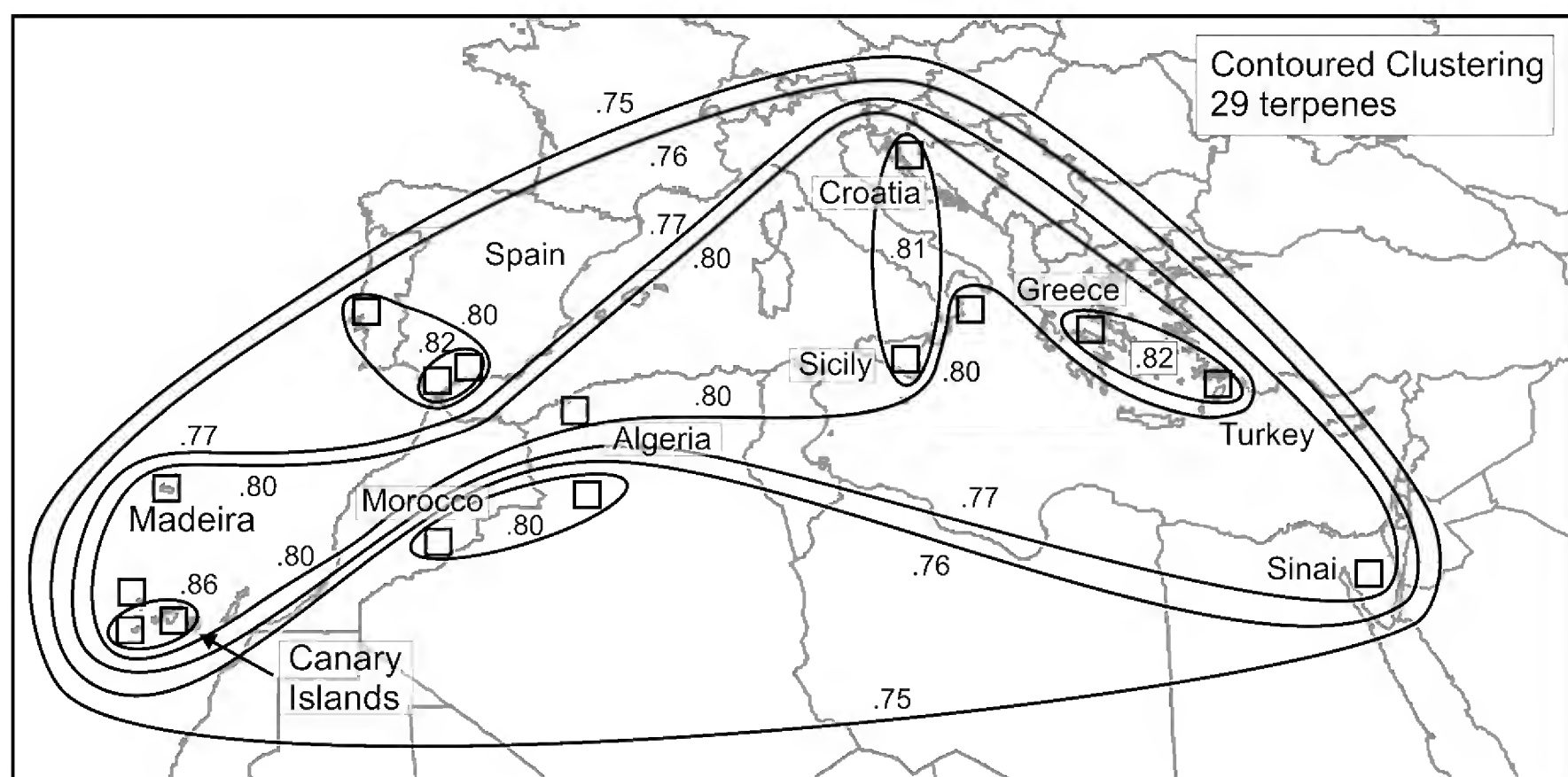


Figure 4. Contoured clustering with similarities based on 29 terpenes.

The pattern of differentiation appears mostly mosaic (Fig. 4), suggesting either a long period of isolation between populations or, perhaps, local selection for terpene profiles.

Initially, it was thought that this study could be done by utilizing oils obtained from herbarium specimens. Several studies (Achak et al. 2008; Adams, 2010; 2011; 2012a; 2012b; 2013a; 2013b; Shanjani et al. 2010) on the effects of leaf drying on volatile leaf oils have shown *Juniperus* terpenes to be quite stable (for up to 2-3 years at room temperature conditions). However, all of these studies utilized care in low temperature air drying of fresh leaves and mild storage conditions. Based on these studies, oils were obtained from herbarium specimens ranging in age from 3 to 12 yrs of storage. The Crotone, Italy provides a good case study because the populations are very well identified by precise GPS coordinates and easy to re-locate and re-sample. Fresh material was collected and the oils from fresh and 12 year old herbarium leaves were analyzed. In contrast to previous studies of dried leaf oils, these oils were very different in composition (Table 2). Although one would expect lower concentration of the most volatile compounds in comparing fresh vs. 12 year old specimens, that was not the case for α -pinene (17.7, 18.3%), but it was true for β -pinene (0.9, 0.4%), myrcene (5.3, 0.2%), δ -2-carene (0.5, 0.0%), α -phellandrene (3.3, 0.3%), and especially for β -phellandrene (24.6, 1.2%). But, some monoterpenes increased from fresh to 12 year old leaves: thuja-2,4-diene (trace, 0.7%), δ -3-carene (0.0, 0.6%), and p-cymene (0.0, 1.1%).

Part of the changes in the oils may be due to oxidation to produce alcohols and ketones. Notice (Table 2) the differences in α -campholenal (0.3, 1.8%), trans-pinocarveol (0.6, 5.4%), trans-verbenol (0.4, 5.4%), trans-pinocamphone (0.0, 0.7%), p-mentha-1,5-dien-8-ol (0.1, 1.7%), myrtenol (0.0, 2.0%), verbenone (0.0, 2.3%) and trans-carveol (0.1, 1.2%).

The large decrease in monoterpenes was correlated with an increase (Table 2) in some of the less volatile diterpenes: manoyl oxide (0.4, 11.9%), abietatriene (trace, 0.4%), trans-totarol (0.8, 2.2%) and trans-ferruginol (trace, 0.3%). However, some diterpenes decreased in the 12 year old specimens: abietadiene (0.2, trace), 4-epi-abietal (0.5%, trace). Several of the sesquiterpenes decreased and others increased between fresh and 12 year old leaves (Table 2)

It appears likely that both oxidation and free radical reactions have occurred in the 12 year old herbarium leaves. This same trend was seen in oils of *J. phoenicea* var. *phoenicea* between fresh and herbarium leaf oils (unpublished). So, in this study, it was not possible to utilize oils from herbarium specimens.

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Table 1. Composition of the leaf oils of *J. turbinata* (*J. phoenicea* var. *turbinata*): Canary Islands, Sinai, Morocco (940 m), Madeira, Turkey (sw coast), Sicily, Tarifa sand dunes, Crotone, Italy and *J. phoenicea* (var. *phoenicea*): phoen-El Pen (El Penon, Spain, low cedrol), phoen Graz (Grazalema, Spain, hi cedrol). Compounds that appear to distinguish taxa are in boldface. Cedarwood oil components are in italics.

KI	Compound	Canary Islands	Sinai	Morocco 940 m	Madeira	Turkey	Sicily	Tarifa sand	s Italy Crotone	phoen-El Pen	phoen-Graz
921	tricyclene	0.2	0.2	0.3	0.1	t	0.1	0.1	t	0.1	t
932	<i>α-pinene*</i>	67.9	52.2	65.4	57.8	32.6	45.3	25.0	17.7	41.2	29.7
945	<i>α-fenchene</i>	0.1	t	0.2	0.1	0.2	0.1	t	t	0.1	t
946	camphene	0.4	0.3	0.6	0.3	0.2	0.3	0.2	0.1	0.1	0.3
953	thuja-2,4-diene	0.1	0.1	0.5	0.2	t	-	-	t	0.1	t
961	verbenene	-	-	-	-	t	-	0.1	-	0.3	t
969	sabinene	0.4	0.1	0.2	0.2	t	t	t	t	0.1	t
974	<i>β-pinene*</i>	1.6	0.8	0.8	1.2	0.8	1.1	1.3	0.9	2.1	1.2
988	<i>myrcene*</i>	2.7	1.9	1.7	3.3	3.6	4.1	6.6	5.3	3.2	1.9
1001	<i>δ-2-carene</i>	t	t	0.2	0.1	t	0.2	0.5	0.5	0.1	t
1002	<i>α-phellandrene*</i>	-	t	-	1.1	1.2	1.6	4.4	3.3	0.7	0.2
1008	<i>δ-3-carene*</i>	0.3	t	2.3	-	4.6	2.0	-	-	1.5	t
1014	<i>α-terpinene</i>	0.1	t	0.1	0.1	t	t	0.3	0.2	0.1	t
1020	p-cymene	0.1	0.3	0.6	0.5	1.1	0.9	1.3	-	0.4	0.2
1024	limonene	1.9	0.7	0.9	0.9	t	1.5	t	t	0.6	0.4
1025	<i>β-phellandrene*</i>	1.2	0.5	0.6	8.0	11.0	13.4	31.5	24.6	4.9	0.6
1044	(E)-β-ocimene	0.2	t	-	0.3	0.2	t	t	t	-	t
1054	<i>γ-terpinene*</i>	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.2	0.2	0.9
1083	fenchone	t	-	1.0	t	-	-	-	-	-	
1086	terpinolene*	0.6	0.5	-	1.0	1.1	0.7	1.8	0.9	0.7	0.4
1095	linalool*	0.4	0.3	0.3	1.0	0.7	0.5	0.1	0.2	1.0	0.2
1100	n-nonanal	0.1	-	-	t	-	-	-	-	-	
1118	cis-p-menth-2-en-1-ol	-	-	-	-	t	0.2	0.6	0.6	0.2	
1122	<i>α-campholenal</i>	0.1	0.4	0.5	0.7	0.7	0.2	0.1	0.3	0.2	t
1135	<i>trans-pinocarveol*</i>	0.1	0.4	0.5	0.7	0.8	0.3	0.5	0.6	0.3	t
1139	C ₁₀ OH, 41,55,81,95,152	-	-	-	-	-	-	-	-	1.4	
1140	<i>trans-verbenol*</i>	0.1	0.4	0.6	0.1	0.5	0.3	0.1	0.4	-	0.2
1141	camphor	-	0.5	1.3	-	-	-	-	-	-	
1158	trans-pinocamphone	-	0.3	0.2	t	-	-	-	-	0.1	
1160	pinocarvone	t	0.2	0.2	t	-	-	-	-	-	
1165	borneol	-	-	-	-	-	-	-	-	0.6	
1166	p-mentha-1,5-dien-8-ol*	0.1	0.2	0.3	0.4	0.5	0.2	0.1	0.1	-	
1172	cis-pinocamphone	-	0.3	0.2	-	-	-	-	-	0.2	
1174	terpinen-4-ol	0.2	0.2	0.3	t	0.2	t	0.2	0.1	0.1	t
1178	naphthalene	0.2	-	-	t	-	t	-	-	t	
1179	p-cymen-8-ol	t	t	-	0.1	t	t	0.4	0.8	0.1	
1186	<i>α-terpineol*</i>	0.2	0.3	-	t	0.6	1.1	0.4	2.4	2.3	t
1195	cis-piperitol	-	-	-	-	0.3	0.1	0.2	0.3	-	
1195	myrtenol	-	0.3	t	0.1	-	t	-	-	0.1	t
1204	verbenone	0.2	0.2	0.3	0.3	-	t	-	-	0.2	
1207	trans-piperitol	-	-	-	-	0.3	t	0.3	0.3	-	
1215	trans-carveol	-	0.2	0.2	0.1	0.3	-	t	0.1	0.1	
1223	citronellol*	t	0.3	1.4	0.1	1.0	0.2	0.6	0.9	0.5	
1233	pulegone	-	0.2	-	-	-	-	-	-	0.1	
1249	piperitone	-	-	-	-	1.0	0.2	0.3	1.9	0.2	
1254	linalyl acetate	-	0.5	-	-	-	-	-	-	-	
1255	(4Z)-decenol*	0.6	-	0.5	0.7	1.8	1.0	0.5	0.5	0.2	t
1257	methyl citronellate	-	0.2	-	-	-	-	-	-	-	
1274	neo-isopulegyl acetate	t	0.3	0.1	0.2	1.2	-	0.8	0.5	-	
1287	bornyl acetate	0.4	-	0.1	0.4	0.5	-	0.2	-	-	
1287	trans-linalool oxide acetate(pyranoid)	-	t	-	-	-	-	0.2	-	-	
1315	(E,E)-2,4-decadienal	0.2	t	t	-	t	0.7	0.3	-	0.3	
1346	<i>α-terpinyl acetate*</i>	0.2	0.2	-	5.0	8.1	4.1	13.1	14.6	0.1	
1346	<i>α-cubebene</i>	0.1	0.2	0.2	-	-	-	-	-	-	
1374	<i>α-copaene</i>	-	0.5	0.1	-	-	-	-	0.2	-	
1387	<i>β-bourbonene</i>	-	0.3	0.1	-	-	-	-	-	-	
1387	<i>β-cubebene</i>	t	0.2	-	-	-	-	-	-	-	

KI	Compound	Canary Islands	Sinai	Morocco 940 m	Madeira	Turkey	Sicily	Tarifa sand	s Italy Crotone	phoen-El Pen	phoen-Graz
1389	β -elemene	-	0.2	-	-	-	-	-	-	0.1	
1410	α-cedrene	-	-	-	-	-	-	-	-	-	1.0
1417	(E)-caryophyllene*	0.6	1.8	0.8	0.9	0.8	1.0	0.1	1.5	1.2	1.3
1429	cis-thujopsene	t	-	0.2	0.2	-	-	-	-	-	0.3
1448	cis-muurolo-3,5-diene	0.5	0.8	0.3	t	-	-	-	-	-	
1452	α -humulene*	0.6	1.3	0.2	0.7	0.6	0.5	-	0.9	-	
1475	trans-cadina-1(6),4-diene	0.6	1.4	0.4	-	t	-	-	0.7	-	
1478	γ -muurolene	-	0.4	0.5	0.1	t	-	-	0.2	-	
1484	germacrene D*	-	2.3	-	-	2.0	2.2	0.2	2.5	0.5	0.3
1493	trans-muurolo-4(14),5-diene	1.2	2.5	0.5	0.1	-	-	-	0.8	-	
1493	epi-cubebol*	0.6	1.2	0.4	0.2	0.5	-	-	0.6	-	
1495	γ -amorphene	-	-	-	-	0.8	0.5	0.1	-	-	
1500	α -muurolene	0.3	0.7	0.3	0.2	0.4	0.2	0.1	0.4	-	
1505	β -bisabolene	-	-	-	-	-	-	-	-	-	0.4
1509	C ₁₅ OH, 41, 55, 81, 161, 220	-	-	0.1	-	0.6	t	0.1	-	0.3	
1512	α -alaskene	-	-	-	-	-	-	-	-	-	0.4
1513	cubebol	1.2	3.2	0.4	0.3	-	-	-	-	-	
1513	γ -cadinene*	1.6	t	-	0.5	0.9	0.3	0.1	1.2	0.1	t
1521	trans-calamenene	-	1.6	-	-	-	-	-	-	-	
1522	δ -cadinene	-	1.1	1.1	-	1.3	0.7	0.4	1.2	0.2	0.2
1528	zonarene	-	0.5	0.2	-	0.2	t	-	-	-	
1533	trans-cadina-1,4-diene	0.2	-	-	-	-	-	-	-	-	
1535	C₁₅OH, 41, 69, 105, 161, 204	-	-	-	-	-	t	0.1	-	1.0	
1544	α -calacorene	-	0.2	-	-	-	-	-	-	-	
1548	elemol*	0.1	0.3	0.7	0.3	0.6	0.4	0.6	0.3	1.8	0.5
1559	germacrene B	-	1.1	-	-	0.5	0.8	0.2	1.1	0.6	0.2
1561	(E)-nerolidol	-	t	0.9	-	-	-	-	-	t	
1574	germacrene-D-4-ol	0.2	-	0.1	0.5	0.3	0.2	0.2	0.1	0.2	
1582	caryophyllene oxide*	0.5	0.8	0.6	0.4	0.7	0.5	0.1	0.5	1.0	
1589	allo-cedrol										1.1
1600	cedrol	-	0.3	-	-	-	-	-	-	-	16.4
1608	humulene epoxide II	0.3	0.4	0.1	0.1	-	-	-	-	-	
1625	C ₁₅ OH, 43, 119, 161, 204, 220	2.3	-	1.2	0.4	-	0.6	0.3	-	0.4	
1627	1-epi-cubenol*	-	3.5	-	-	1.5	-	-	1.4	-	
1630	γ -eudesmol	-	t	-	-	t	t	0.1	t	0.2	t
1638	epi- α -cadinol	0.5	0.2	0.2	0.2	0.2	t	0.1	0.1	0.2	t
1638	epi- α -muurolol	0.5	0.2	0.2	0.3	0.2	t	0.2	0.1	0.1	t
1649	β -eudesmol	-	t	0.2	0.2	0.2	0.3	0.2	0.1	0.4	
1652	α-eudesmol*	-	0.2	0.2	0.9	0.4	0.4	0.2	0.4	0.3	t
1652	α-cadinol	1.0	0.2	0.2	-	0.4	0.3	0.3	-	0.3	t
1687	eudesma-4(15),7-dien-1-β-ol*	0.7	0.3	t	0.6	0.6	0.7	-	0.3	0.1	
1688	shyobunol*	-	0.6	0.5	1.0	1.0	1.0	0.8	0.6	1.5	0.5
1715	(2Z,6E)-farnesol	-	-	0.1	-	-	-	-	-	1.2	
1968	sandaracopimara-8(14), 15-diene	-	t	-	-	t	t	0.1	t	0.1	0.2
1978	manoyl oxide*	1.1	2.6	2.6	-	0.9	1.2	0.4	0.4	22.0	32.9
2009	epi-13-manoyl oxide	-	-	0.1	-	-	-	-	-	0.1	0.2
2055	abietatriene	0.1	t	-	0.3	0.3	0.4	t	t	0.1	0.4
2087	abietadiene	t	0.2	-	0.4	0.4	t	0.1	0.2	0.1	t
2298	4-epi-abietal	0.2	0.5	0.1	0.4	0.3	t	t	0.5	0.2	0.2
2314	trans-totarol*	0.4	0.2	0.1	2.1	1.0	t	0.2	0.8	0.2	1.9
2331	trans-ferruginol	-	t	-	0.2	0.2	t	t	t	-	0.3

KI = linear Kovats Index on DB-5 column. *Used in numerical analyses. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of the oils from fresh leaves and 12 yr old herbarium leaves from Croton, Italy. Compounds with large changes in concentration are in boldface.

KI	Compound	fresh leaves	12 yr old leaves
921	tricyclene	t	0.1
932	α -pinene	17.7	18.3
945	α -fenchene	t	0.1
946	camphene	0.1	0.2
953	thuja-2,4-diene	t	0.7
969	sabinene	t	0.3
974	β-pinene	0.9	0.4
988	myrcene	5.3	0.2
1001	δ-2-carene	0.5	-
1002	α-phellandrene	3.3	0.3
1008	δ-3-carene	-	0.6
1014	α -terpinene	0.2	-
1020	p-cymene	-	1.1
1024	limonene	t	0.3
1025	β-phellandrene	24.6	1.2
1044	(E)- β -ocimene	t	-
1054	γ -terpinene	0.2	t
1086	terpinolene	0.9	0.7
1095	linalool	0.2	0.3
1118	cis-p-menth-2-en-1-ol	0.6	0.2
1122	α-campholenal	0.3	1.8
1135	trans-pinocarveol	0.6	5.4
1140	trans-verbenol	0.4	5.4
1158	trans-pinocamphone	-	0.7
1166	p-mentha-1,5-dien-8-ol	0.1	1.7
1174	terpinen-4-ol	0.1	0.4
1178	naphthalene	-	0.4
1179	p-cymen-8-ol	0.8	1.1
1186	α-terpineol	2.4	0.7
1195	cis-piperitol	0.3	-
1195	myrtenol	-	2.0
1204	verbenone	-	2.3
1207	trans-piperitol	0.3	-
1215	trans-carveol	0.1	1.2
1223	citronellol	0.9	0.6
1249	piperitone	1.9	0.6
1254	linalyl acetate	-	0.9
1255	(4Z)-decenol	0.5	-
1274	neo-isopulegyl acetate	0.5	0.3
1287	trans-linalool oxide acetate(pyranoid)	-	0.1
1315	(E,E)-2,4-decadienal	-	0.1
1346	α-terpinyl acetate	14.6	6.1
1374	α -copaene	0.2	t
1387	β -bourbonene	-	t

KI	Compound	fresh leaves	12 yr old leaves
1389	β -elemene	-	0.2
1410	α -cedrene	-	t
1417	(E)-caryophyllene	1.5	0.2
1448	cis-muurola-3,5-diene	-	0.2
1452	α-humulene	0.9	0.1
1475	trans-cadina-1(6),4-diene	0.7	0.2
1478	γ-muurolene	0.2	0.5
1484	germacrene D	2.5	t
1493	trans-muurola-4(14),5-diene	0.8	0.4
1493	epi-cubebol	0.6	-
1500	α -muurolene	0.4	0.4
1513	cubebol	-	1.0
1513	γ-cadinene	1.2	-
1521	trans-calamenene	-	2.0
1522	δ-cadinene	1.2	-
1528	zonarene	-	0.1
1533	trans-cadina-1,4-diene	-	0.1
1544	α-calacorene	-	0.6
1548	elemol	0.3	0.9
1559	germacrene B	1.1	t
1574	germacrene-D-4-ol	0.1	-
1582	caryophyllene oxide	0.5	4.7
1600	cedrol	-	1.0
1608	humulene epoxide II	-	2.8
1627	1-epi-cubenol	1.4	2.1
1630	γ-eudesmol	t	0.6
1638	epi- α -cadinol	0.1	t
1638	epi- α -muurolol	0.1	t
1649	β-eudesmol	0.1	0.7
1652	α-eudesmol	0.4	1.1
1687	eudesma-4(15),7-dien-1-β-ol	0.3	1.6
1688	shyobunol	0.6	-
1968	sandaracopimara-8(14), 15-diene	t	-
1978	manoyl oxide	0.4	11.9
2055	abietatriene	t	0.4
2087	abietadiene	0.2	t
2298	4-epi-abietal	0.5	t
2314	trans-totarol	0.8	2.2
2331	trans-ferruginol	t	0.3

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

**Importance of mass and light levels for germination of achenes of *Coreopsis tinctoria* Nutt.
(coreopsis, goldenwave, Asteraceae)**

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ABSTRACT

Coreopsis tinctoria Nutt. (coreopsis, goldenwave, Asteraceae) is a spring and early summer flowering annual found throughout most of North America. Its apparent habitat is primarily vegetation gaps or patches in disturbed grasslands and woodlands. It was hypothesized that achene mass and light levels were important for achene germination. Achene mass, determined by seed buoyancy, was used as a surrogate to determine viability. All collected achenes were presumed viable. To test the effectiveness of achene mass as a surrogate for determining seed viability, mean germination of high mass (non-buoyant) achenes was compared to mean germination of low mass (buoyant) achenes. The role of light level was investigated by germinating collected achenes at three light levels: 121.50, 0.01, and 0.00 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The float test correctly identified viable achenes 80% of the time. There were significantly fewer germinations (5 ± 2 , mean $\pm$ standard deviation, 20%) of the low mass achenes than for the high mass achenes (21 ± 4 , 84%), indicating that achene mass was a fast and easy surrogate for determining *C. tinctoria* achene viability. The mean germination of achenes in the light treatments was 84% (21 ± 4) with no significant differences in the mean number of achenes that germinated in the three light treatments. In addition, 56% of the achenes germinated four days after placement into a moist environment and 84% germinated in eight days, suggesting a lack of dormancy. Published on-line www.phytologia.org *Phytologia* 96(3): 159-166 (July 1, 2014). ISSN 030319430

KEY WORDS: achene mass, seed buoyancy, float test, seed germination, achene germination, *Coreopsis tinctoria*, coreopsis, goldenwave, plains coreopsis, calliopsis, golden tickseed, seed/achene viability

Successful establishment of a plant from seed requires favorable environmental conditions for seed germination (Fenner 1985). Typical environmental conditions required for germination include appropriate temperature, soil moisture, and oxygen levels. In some cases, light level, photoperiod, and the red/far-red light ratios are also necessary for germination (Baskin and Baskin 1998). Factors that control seed germination can give insights into the habitat and growth requirements of a species (Van Auken 2013). Seed mass, measured by buoyancy in water, is a common, non-destructive, rapid technique used to estimate the viability of collected seeds (Paynter and Dixon 1990, Gribko and Jones 1995, Dalgleish et al. 2012). However, its use has not been tested with *Coreopsis tinctoria* achenes (seeds). Many non-dormant seeds need light for germination, and it has been reported that *C. tinctoria* achenes do require certain light levels for germination (Baskin and Baskin 1998).

Coreopsis tinctoria (Fig. 1) is commonly known as goldenwave, plains coreopsis, calliopsis, or golden tickseed (Native Plant Information Network 2009). It is an early spring and early summer flowering annual in the Asteraceae family that reaches heights between 250 and 1200 cm (Correll and Johnston 1979, Native Plant Information Network 2009), and is widely distributed throughout most of the

lower 48 United States, parts of southern Canada, and northern Mexico (Fig. 2) (Strother 2006, USDA 2012). It can tolerate various soil types, but seems to prefer moist sandy or clayey soils (Enquist 1987, Strother 2006). It is not a good competitor (Elliott and Van Auken 2014) similar to other species of *Coreopsis* (Folgate and Scheiner 1992) and appears to grow best in open grassland patches, disturbances, or gaps. It was hypothesized that achene mass and light levels were important for achene germination in *C. tinctoria*.

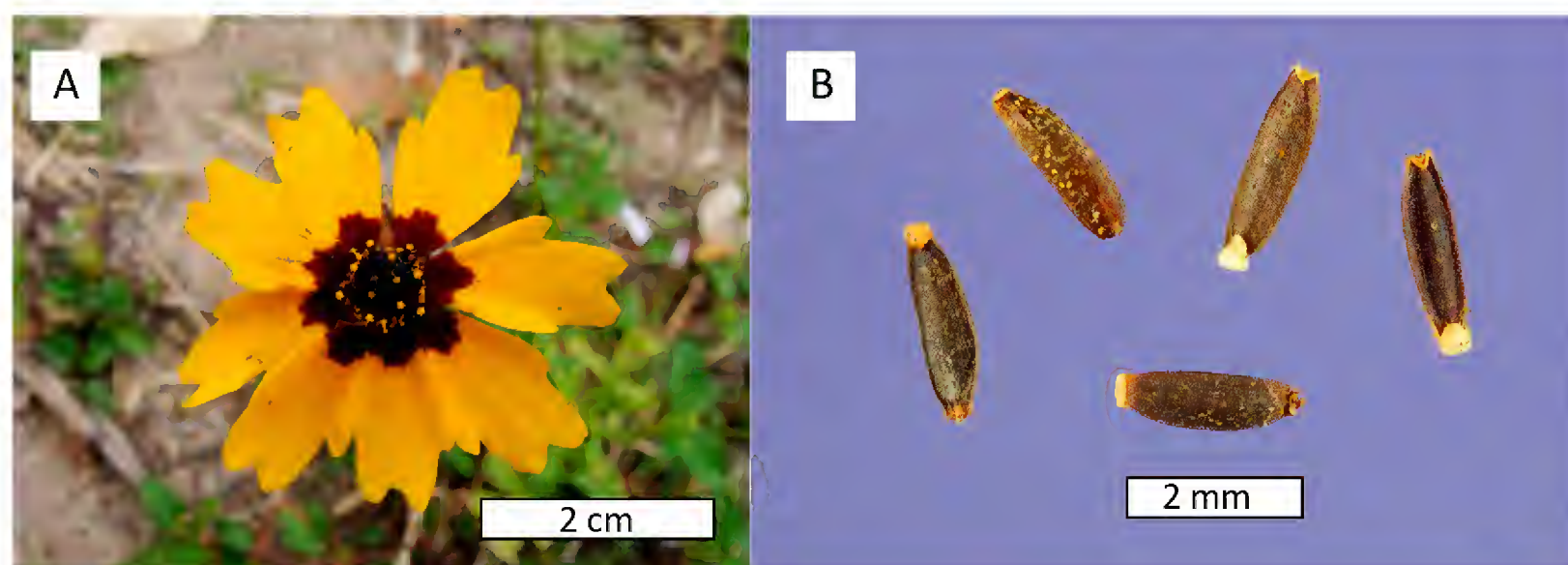


Figure 1. *Coreopsis tinctoria* flower head (A – Photograph by K. C. Eddy, senior author) and achenes (B – Photograph by Steve Hurst (USDA 2012)).

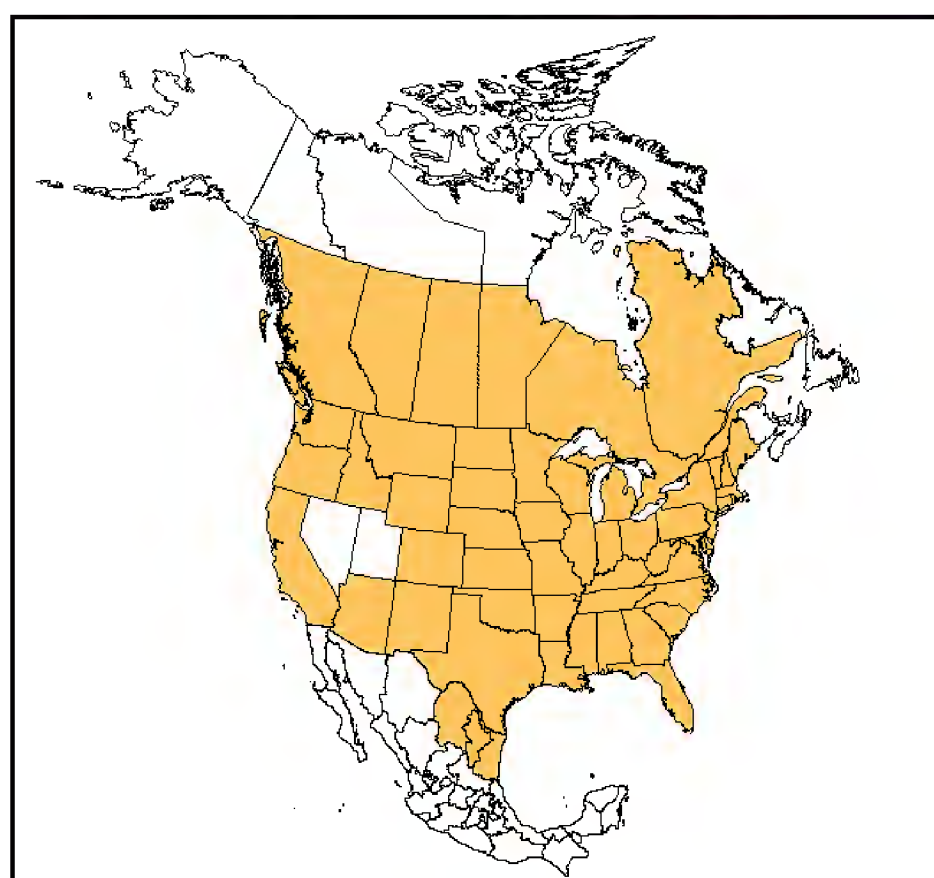


Figure 2. Distribution (shaded area) of *Coreopsis tinctoria* in the United States, Canada, and northern Mexico (Strother 2006). Albers equal-area conic projection.

MATERIALS AND METHODS

Mature *Coreopsis tinctoria* seed heads were harvested from various locations in northwestern Bexar County, Texas (29°35' N, 98°37' W), in May of 2011. Seed heads were dried and stored at room temperature in paper sacks for six months prior to starting the experiments. Seed heads were then crushed in a resealable plastic storage bag and sieved with a 2 mm sieve to remove most chaff from the achenes (seeds). Achenes were then removed from remaining chaff by picking with tweezers or a metal spatula.

Achene mass (buoyancy) (Arrillaga et al. 1992, Elliott 1999) was tested as a potential surrogate for viability of collected achenes. One half ml of detergent was added to 400 ml deionized water to reduce surface tension. Thirty-five batches of 30 achenes each were used to separate low mass and high mass achenes in the water-detergent solution. Following separation of the low mass and high mass achenes, they were rinsed in deionized water, dried, and then a portion of each group was tested for ability to germinate.

The germination test consisted of two treatments (low mass and high mass achenes), each with eight replicates. Each replicate consisted of twenty five achenes placed on a 700 mm diameter Whatman® #4 qualitative filter paper in a petri plate. One ml of deionized water was placed on the filter paper; the plates were covered, placed in resealable plastic storage bags to prevent evaporation, and placed in the laboratory at low light and at approximately 25° C. Throughout the experiment, 1 ml of deionized water was added every two days, if needed, to keep the filter paper moist. Achenes were checked for germination every other day for two weeks. Germination occurred when the radicle emerged and was >1 mm long (Baskin and Baskin 1998, Elliott 1999). Achenes that germinated were counted and removed from the petri plates. The plates were then replaced in the plastic storage bags which were resealed. The number of achenes that germinated and the length of time required for germination were recorded. The mean total number of achenes that germinated was compared using a Wilcoxon rank-sum test. The number that germinated in time was compared using a Kruskal-Wallis one-way non-parametric ANOVA followed by pairwise comparisons using the Wilcoxon rank-sum test (Sall et al. 2012).

Achene germination was also determined at three light levels. Light levels were 121.50 $\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$, 0.01 $\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$, and 0.00 $\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$. Light levels were measured using a Li-Cor® LI-190 Quantum Sensor attached to a Li-Cor® LI-1000 Data Logger. Temperatures were approximately 25° C. Every other day, for two weeks, the achenes were checked for germination. Germination occurred when the radicle emerged and was >1 mm long (Baskin and Baskin 1998, Elliott 1999). Those that germinated were counted and removed from the petri plates and the plate was replaced in the treatment as above. The number of achenes that germinated and the time required for germination were recorded. The mean number of germinations in the light treatments was compared using a one-way non-parametric ANOVA and pairwise comparisons, as above (Sall et al. 2012).

Because there were no significant differences in the number of germinations between the light treatments, results from these three treatments were pooled to compare germinations through time. The number of achenes that germinated per two days was plotted as was the sum of the mean number of achenes that germinated. In addition, the mean and standard deviation of the total number of achenes that germinated in each treatment was plotted as a bar graph.

RESULTS

In the achene mass experiment, 52% of all collected achenes germinated; 20% (5 ± 2 , $\bar{x} \pm \text{SD}$) of the low mass achenes, which was significantly fewer than the 84% (21 ± 4) of high mass achenes (Fig. 3, Wilcoxon rank-sum, $H=11.41$, $P=0.0007$). On day 4, 3 ± 2 low mass achenes germinated, significantly more than on any other day (Fig. 4; one-way non-parametric ANOVA, $H=25.67$, $P<0.0001$, Kruskal-Wallis followed by Wilcoxon rank-sum). Significantly more high mass achenes germinated on day 4 (18 ± 3) than on any other day, and significantly more high mass achenes germinated on day 6 (3 ± 3) than on days 2, 8, and 10 (Fig. 4; one-way non-parametric ANOVA, $H=33.40$, $P<0.0001$, Kruskal-Wallis followed by Wilcoxon rank-sum). There were no further low mass or high mass achene germinations after day 6 (Figs. 4 & 5).

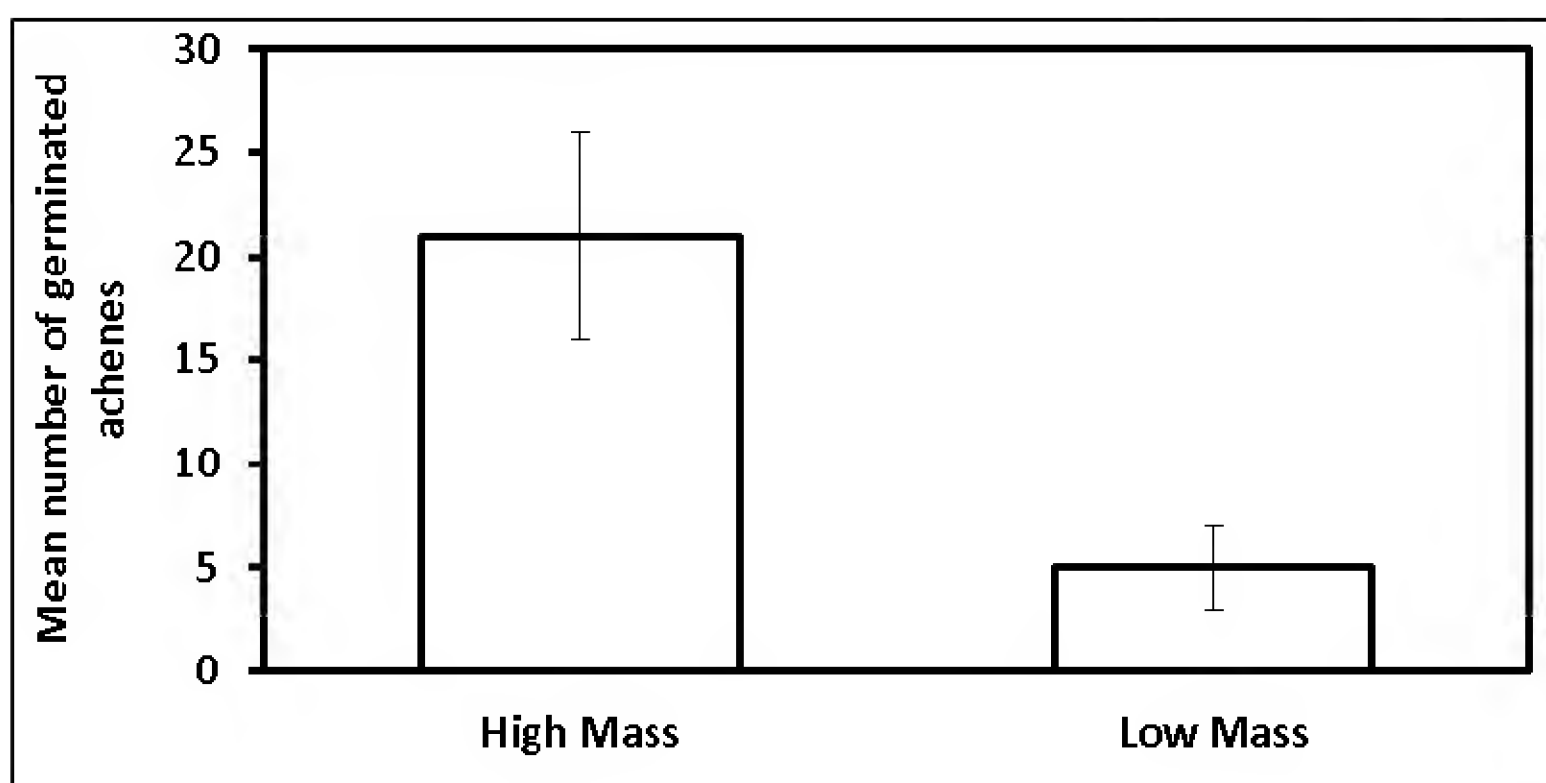


Figure 3. Comparison of the mean total number of germinated achenes after separation by mass. Of the high mass achenes, 84% germinated (21 ± 4), significantly more than the 20% of low mass achenes that germinated (5 ± 2) (Wilcoxon rank-sum one-way non-parametric ANOVA, $H=11.41$, $P=0.0007$). Error bars indicate $\pm$ one standard deviation of the mean.

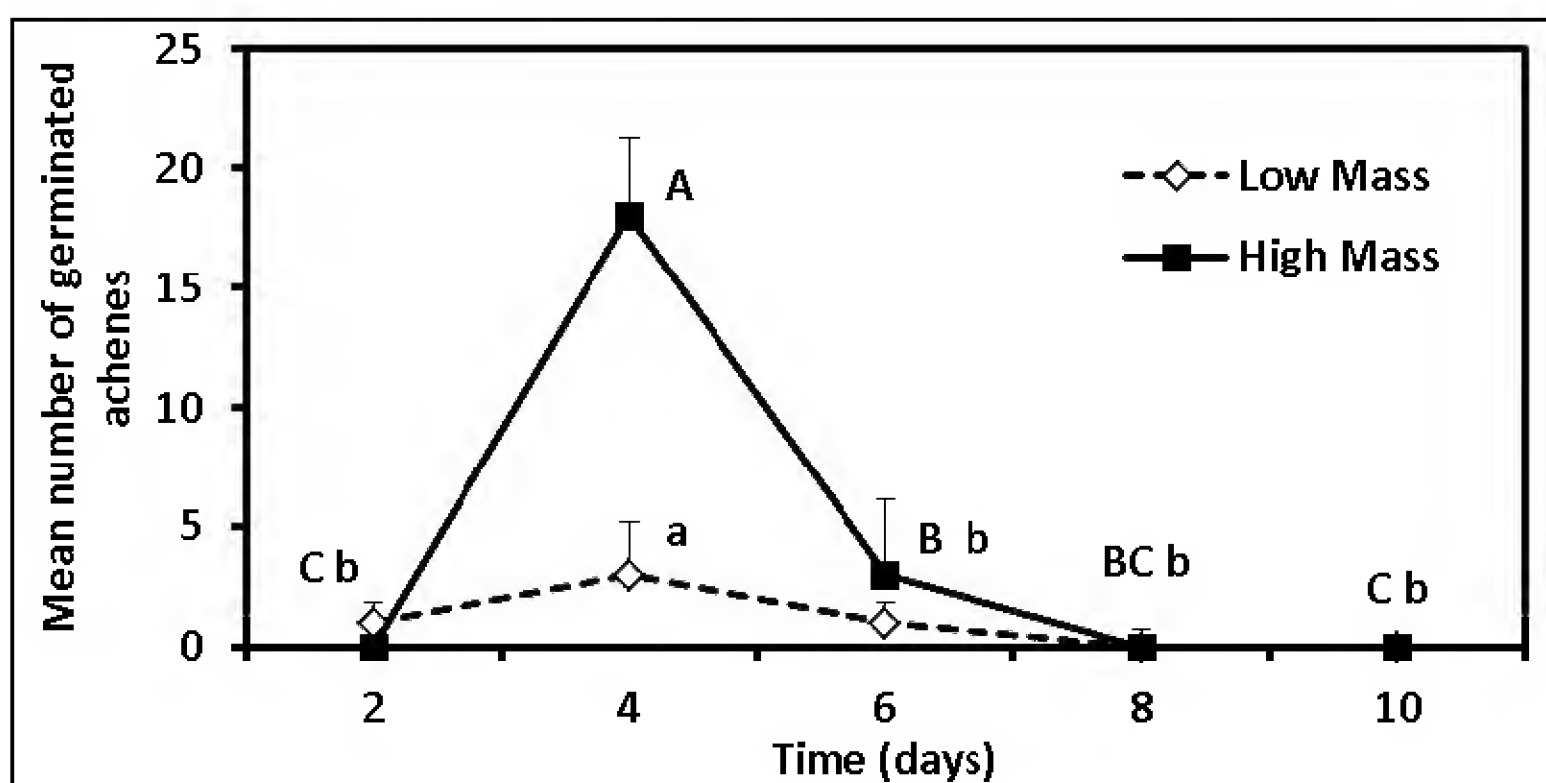


Figure 4. Comparison of low mass and high mass achene germinations per two days (error bars indicate $\pm$ one standard deviation of the mean). Significantly more low mass or high mass achenes germinated on day 4 than on any other day (Kruskal-Wallis one-way non-parametric ANOVA, $H=25.67$, $P<0.0001$ and $H=33.40$, $P<0.0001$, respectively, Wilcoxon rank-sum pairwise comparison). For either type of achene, the same upper case (high mass) or lower case (low mass) letters indicate no significant difference.

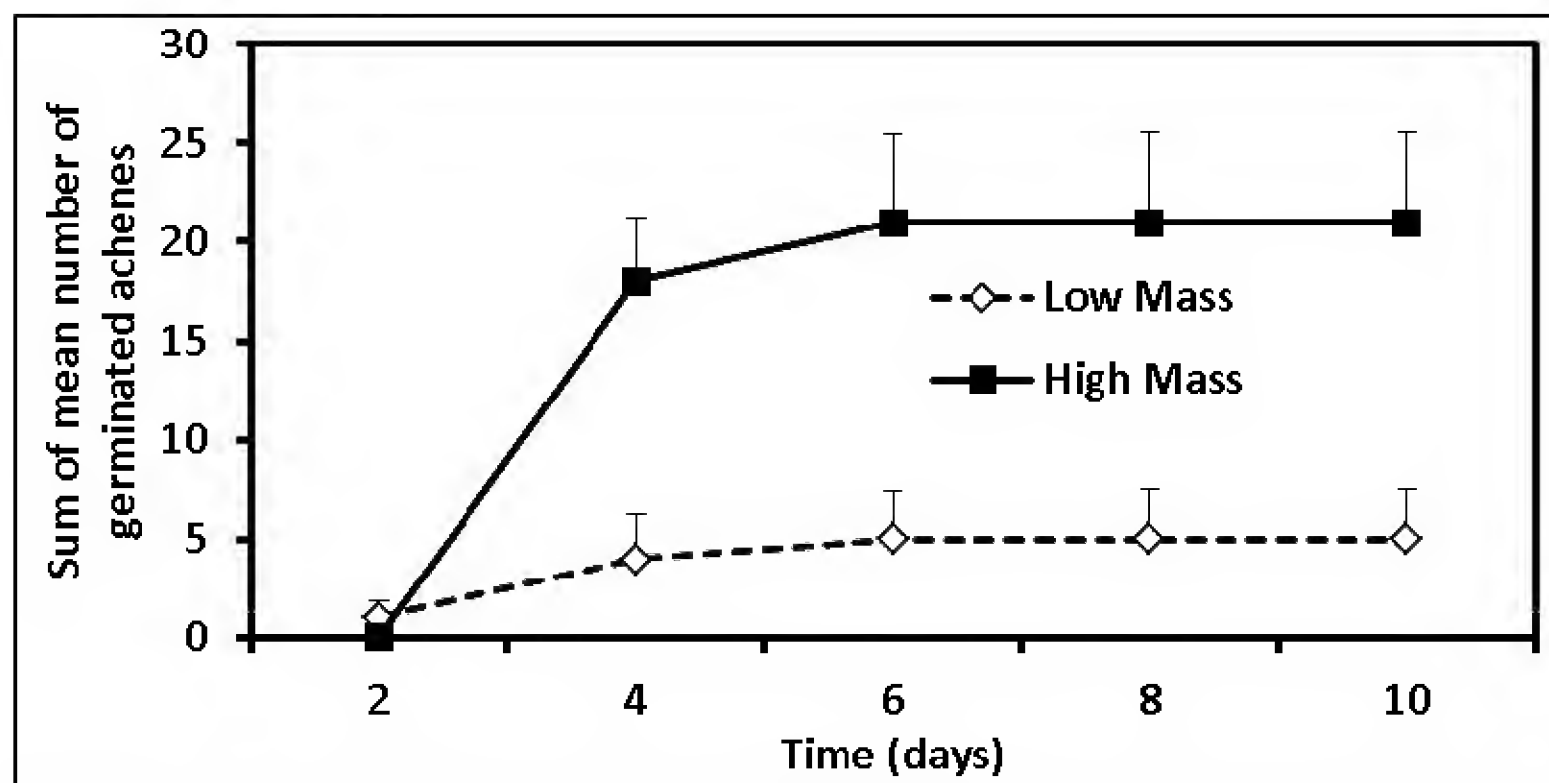


Figure 5. Comparison of the sum of the mean of low mass and high mass achene germinations per two days (error bars indicate + one standard deviation of the mean). Out of a total of 25 seeds used in each replicate, 5 ± 2 low mass achenes germinated and 21 ± 4 high mass achenes germinated. In both cases, there were no further germinations after day six.

In the light treatment experiment, the mean number of germinated achenes at $125.50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (21 ± 4) was not significantly different than the 20 ± 2 achenes germinated at $0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ or the 20 ± 3 achenes germinated at $0.00 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 6, one-way nonparametric ANOVA, $H=1.77$, $P=0.4121$, Kruskal-Wallis). Examination of the pooled results shows that significantly more achenes germinated on day 4 (14 ± 4) than any other day, and significantly more germinated on day 6 (6 ± 4) than days 2, 8, or 10 (Fig. 7, one-way non-parametric ANOVA, $H=100.91$, $P<0.0001$, Kruskal-Wallis followed by Wilcoxon rank-sum). No more achenes germinated after day 8 (Fig. 8). Overall, approximately 84% of all tested achenes in the light treatments germinated or conversely 16% did not germinate and appeared nonviable.

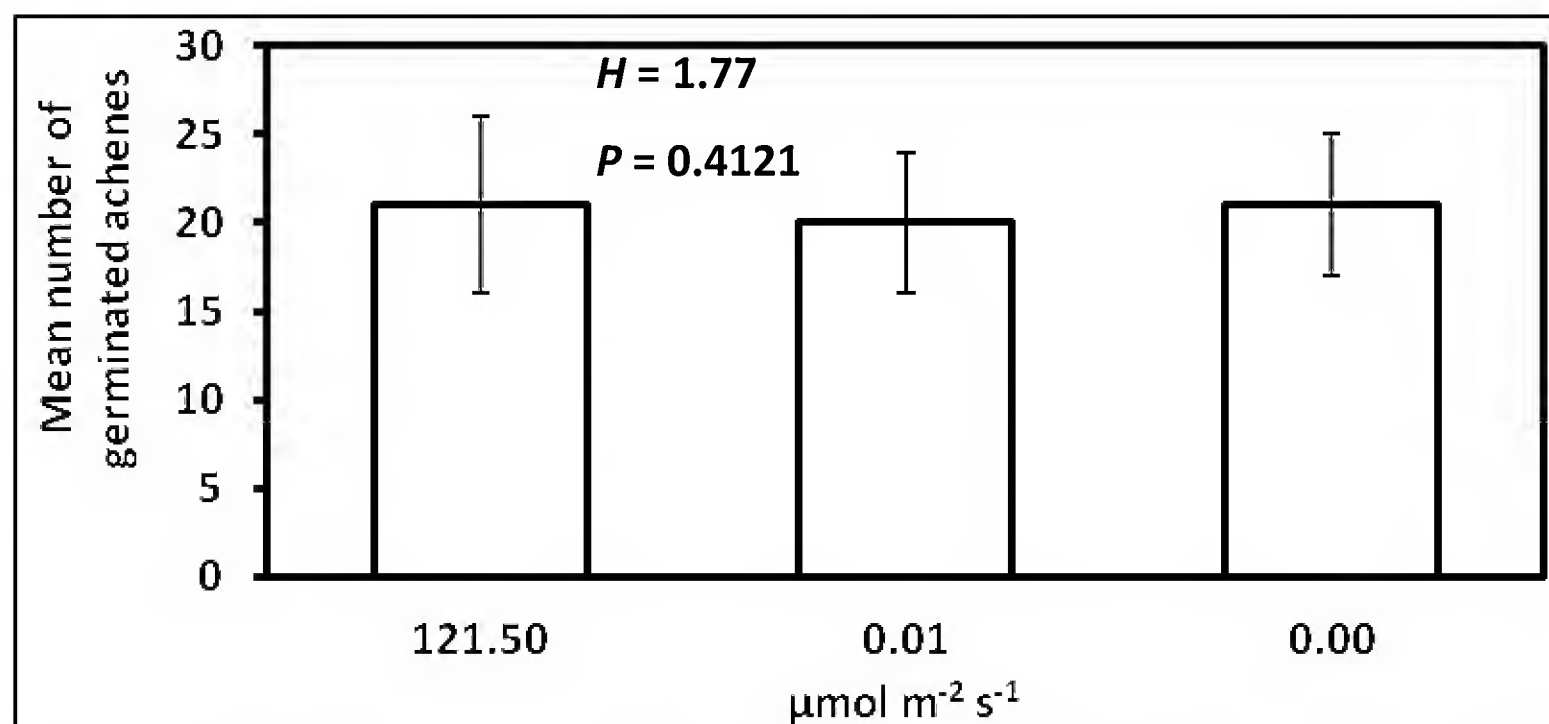


Figure 6. Comparison of the mean number of germinated achenes in each light treatment. There were no significant differences in mean number of germinations between light treatments (Kruskal-Wallis one-way non-parametric ANOVA, $H=1.77$, $P=0.4121$). Error bars indicate $\pm$ one standard deviation of the mean.

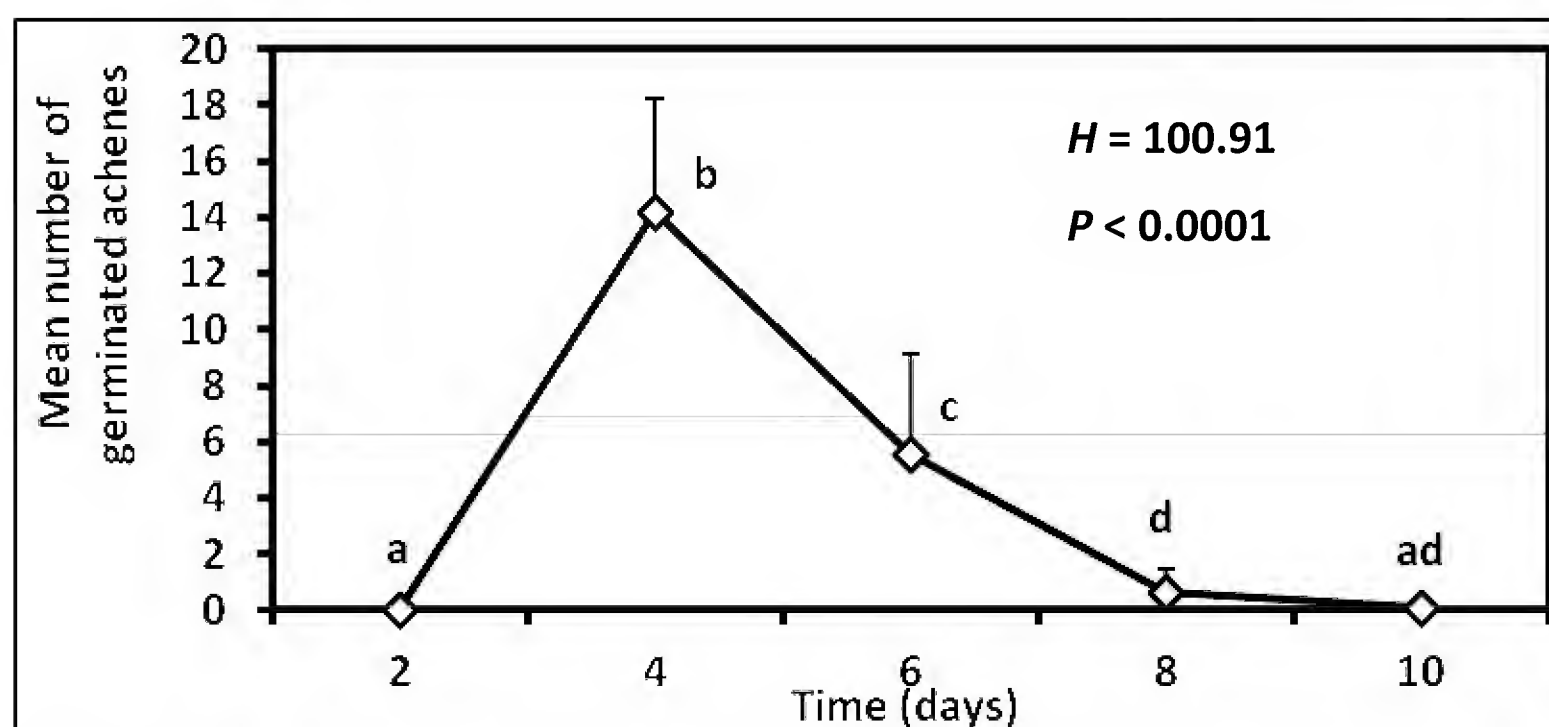


Figure 7. Pooled mean number of germinated achenes in the light treatments per two days. Sixty six percent of the achenes germinated through day 4 (14 ± 4), significantly more than any other day (Kruskal-Wallis one-way non-parametric ANOVA, $H=100.91$, $P<0.0001$, Wilcoxon rank-sum pairwise comparison). Different letters indicate a significant difference. Error bars indicate + one standard deviation of the mean.

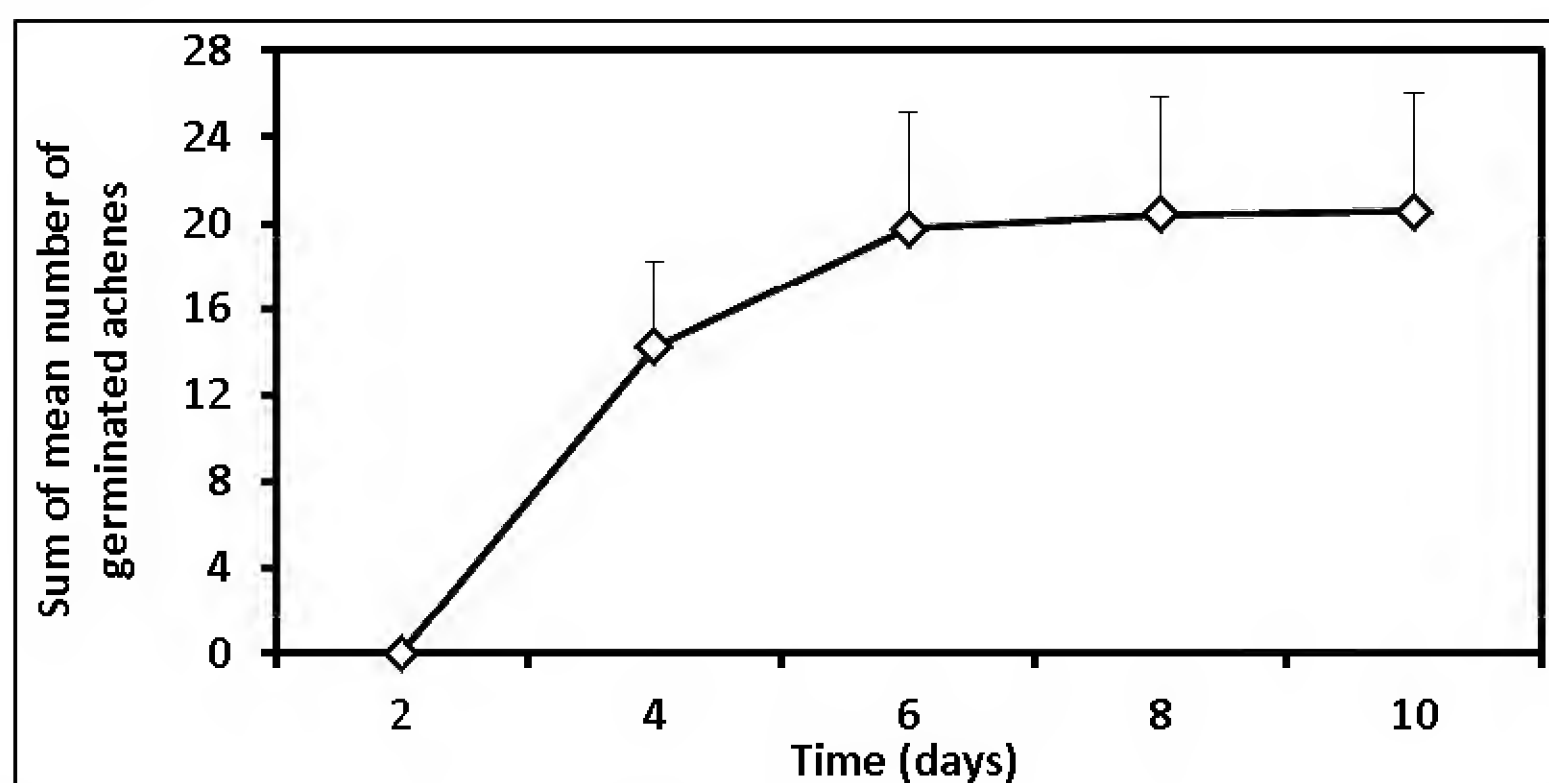


Figure 8. Pooled sum of mean number of achenes germinated per two days. After 8 days, 21 ± 4 achenes had germinated. There were no further germinations after 8 days. Error bars indicate + one standard deviation of the mean.

DISCUSSION

For a plant to be successful, its seeds must germinate when environmental conditions favor the survival of seedlings and are conducive to their growth and reproduction (Fenner 1985, Kettenring et al. 2006). By understanding the germination requirements of seeds, seedlings could be available for experimentalists to do ecological, physiological, or genetic studies and to better understand species interactions in the environment, including competition (Smith and Smith 2012). Interspecific competition has been shown to negatively affect the growth of *C. tinctoria* (Eddy 2013, Elliott and Van Auken 2014).

and *C. lanceolata* (Folgate and Scheiner 1992) and determine where *C. tinctoria* occurs by limiting it to physical or temporal gaps (Eddy 2013, Elliott and Van Auken 2014).

Of all collected achenes, 52% germinated, comparable to 48 – 80% for *C. lanceolata* (Norcini and Aldrich 2007), and 69% for *C. leavenworthii* (Kabat et al. 2007). Buoyancy, or the float test, commonly used in horticulture to distinguish viable and nonviable seeds or achenes (Czarnota et al. 2003), appears to be a reasonable, fast, and simple method for determining viability of *C. tinctoria* achenes, with only 20% of the viable achenes misidentified as nonviable. Seed morphology and predation may be factors that affect the usefulness of flotation testing for estimating seed viability. Comparing flotation testing and seed x-raying as viability estimation techniques, the float test misidentified chestnuts damaged by seed predation 50% of the time (Dalglish et al. 2012). However, for acorns the test was correct 91.6% of the time (Gribko and Jones 1995). Flotation testing may not be appropriate for plants that would use buoyancy as a method of seed dispersal, as is the case with some wetland plants (van den Broek et al. 2005).

Light levels and photoperiod have been shown to be important for germination in various members of the family Asteraceae (Meyer et al. 1990, Baskin and Baskin 1998, Elliott 1999). However, in the current study variation in low light levels was not a factor in *C. tinctoria* germination. In a study of *C. leavenworthii* that incorporated photoperiod, exposure to light significantly increased achene germination, while buried achenes did not germinate but became part of the seed bank, suggesting they would have to be brought to the surface by some disturbance before they would germinate (Kabat et al. 2007). The apparent lack of a light effect combined with rapid germination (most achenes germinated by day 4, Fig. 6) suggests that *C. tinctoria* achenes are not dormant. This may allow *C. tinctoria* to germinate as soon as environmental temperature and soil moisture conditions become favorable for growth, giving *C. tinctoria* a growth advantage in gaps or patches in C₄ grasslands where competition for resources may be very intense (Elliott 1999, Smith and Smith 2012, Eddy 2013). A light requirement has been reported for *C. tinctoria* germination (Baskin and Baskin 1998), but not in this experiment, thus further research is needed to clarify the role of light levels in *C. tinctoria* germination.

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**Environmentally induced variation in the leaf volatile terpenes of *Taxodium distichum* (L.) Rich.
(Cupressaceae)**

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ABSTRACT

Volatile leaf oils of *Taxodium distichum*, from two clonal lines, 478-17 and 492-23, were analyzed from test plots in Arkansas, Florida, Iowa, Kansas, and College Station and El Paso, Texas. ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 478-13 revealed one highly significant difference (bornyl acetate) and five significantly different variables (oil yield, α -pinene, terpinolene, caryophyllene oxide, and humulene epoxide II) among the test plots. Oil yield was significantly higher in the El Paso plot (10.1 mg/g) and decreased to the lowest level (7.0 mg/g) in Florida. The yields of α -pinene, bornyl acetate, caryophyllene oxide and humulene epoxide were also highest in the El Paso plot. ANOVA of 478-17 components on a % total oil basis showed a different pattern with four compounds being highly significantly different among plots (terpinolene, α -terpineol, (E)-caryophyllene, α -humulene) and two significantly different (bornyl acetate, humulene epoxide II). The major oil component, α -pinene, was not significantly different, ranging from 78.50% (College Station) to (64.43% (Arkansas). ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 492-23 revealed only one significant difference (eudesma-4(15),7-dien-1- β -ol) among the test plots, being highest in Arkansas and lowest in Iowa. ANOVA of 492-23 components on a % total oil basis showed considerably greater variation with six compounds being highly significantly different among plots (α -pinene, β -pinene, (E)-caryophyllene, α -humulene, α -cadinol, eudesma-4(15),7-dien-1- β -ol) and one significantly different (germacrene D). The highest percent of α -pinene, the major component, was in Kansas (74.37%) and the lowest in Florida (59.40%). PCA did not find high correlations between growth and edaphic variables, and oil yields or oil components. Published on-line www.phytologia.org *Phytologia* 96(3): 167-177 (July 1, 2014). ISSN 030319430

KEY WORDS: *Taxodium distichum*, terpenes, leaf essential oil, environmental induced differences.

Taxodium Rich. is a small genus in which one to three species have been recognized. Britton (1926), Dallimore & Jackson (1966) and Rehder (1940) all recognized three species: bald cypress, *T. distichum* (L.) Rich., pond cypress, *T. ascendens* Brongn. and Montezuma or Mexican bald cypress, *T. mucronatum* Ten. Watson (1985) treated *T. ascendens* as *T. d.* var. *imbricarium* (Nutt.) Croom. Both Farjon (2005) and Eckenwalder (2009) recognized *T. distichum*, *T. d.* var. *imbricarium* and *T. mucronatum*. Denny (2007) treated the genus as monotypic with one species, *T. distichum* and two varieties: var. *imbricarium* and var. *mexicanum* (Carr.) Gord. (= *T. mucronatum*). Denny (2007) and Denny and Arnold (2007) give a lucid discussion of the historical nomenclature of the genus. Recently, Adams et al. (2012a) examined all three putative species using DNA sequences (3547 bp) of nrDNA and three cp regions (petN-psbM, trnS-trnG, ycf-psbA) and concluded that *Taxodium* was best treated as a monotypic genus with three varieties, var. *distichum* (L.) Rich., var. *imbricarium* (Nutt.) Croom and var. *mexicanum* (Carr.) Gord.

Adams et al. (2012b) reported on geographical variation in the leaf volatile terpenes of the three varieties and reviewed the literature on *Taxodium* leaf oils. A study of seasonal variation (Adams, 2012) in the leaf oils of *T. distichum* (near Amarillo, TX) revealed that oil yield increased in new leaves (April, 3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall (July - Oct; 2.94 - 2.32 ns). The major component, α -pinene, exhibited no significant variation on a percent total oil basis, but did differ on a mg/g DM basis. Interestingly, all the other components, besides α -pinene, varied significantly on a percent total oil basis. There was no evidence that *Taxodium* catabolizes terpenes as energy for winter storage as found in some deciduous angiosperms.

There are no literature reports on the environmental plasticity of terpenes in *Taxodium*. The purpose of the present study is to report on environmentally induced variation in the leaf volatile terpenes of *Taxodium distichum* from two clonal lines (478-17 and 492-23), grown in various test plots ranging from Iowa to Texas to Florida.

MATERIALS AND METHODS

Plant material: *Taxodium distichum* Clones 478-17 and 492-23 were a part of a larger replicated trial (Arnold et al., 2012) encompassing 24 clones on ten sites representing north – south and east – west transects across the central and southern United States. The goal of these trials were to evaluate phenotypically elite clones which had been selected from preliminary trials conducted at three sites using seedlings of provenances collected from the central and western portion of the native range of *Taxodium distichum*. From the hundreds of seedlings grown from the provenance collections on three preliminary Texas test sites, located in Dallas, Overton, and College Station, 24 advance clonal selections were made on the basis of their aesthetic properties and subsequently pared to 16 selections based on their adventitious rooting potential during vegetative propagation. These final selections were then propagated for testing on a larger number of sites in a wider range of environments. Clones 478-17 and 492-23 were among these advanced selections. Clone 478-17 was derived from seed collected in Iberia Parish, Louisiana (29°48'0"N, 91°47'24"W), whereas clone 492-23 was derived from seed collected on the Mississippi River in Louisiana (31°33'36"N, 91°26'24"W). Three vegetatively propagated ramets of each of these clones were grown in 2.3 L containers filled with a pine bark based substrate and then shipped to each of the test sites at Ames, IA, College Station, TX, El Paso, TX, Fayetteville, AR, Haysville, KS, and Quincy, FL in spring 2009. Trees were planted in a completely randomized design on 2 m within row and at least 3 m between row spacings. Initial measurements were taken at planting and then again at the beginning and end of each growing season. Growth and aesthetic data collected during each of the three

years included tree height, trunk diameter at 15 cm above the root collar, proportion of the canopy exhibiting chlorotic or necrotic foliage, winter dieback, and cold damage ratings. The tissue samples for volatile oils were derived from the trees in these plantings during the fourth growing season following planting. All leaf samples were collected between 9 and 11 am, local time, on July 16 or 17 and immediately shipped on ice for extraction. Voucher specimens are deposited in the Herbarium, Texas A & M University (for the plot materials).

Isolation of Oils - Fresh leaves (200 g), to which 2 mg, methyl decanoate (internal standard) was added to the leaves after they were placed into the circulatory Clevenger-type apparatus, were steam distilled for 2 h (Adams, 1991). Oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. Extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. Oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software and the internal standard amounts.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the taxa by the Gower metric (Gower, 1971). ANOVA and SNK (Student-Newman-Keuls') multiple range tests follows the formulation of Steel and Torrie (1960). Principle coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principle Components Analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

Clone 478-13

ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 478-13 revealed one highly significant difference (bornyl acetate) and five significantly different variables (oil yield, α -pinene, terpinolene, caryophyllene oxide, and humulene epoxide II) among the test plots (Table 1). Oil yield was significantly higher in the El Paso plot (10.1 mg/g) and decreased to the lowest level (7.0 mg/g) in Florida (Table 1). The yields of α -pinene, bornyl acetate, caryophyllene oxide and humulene epoxide were also highest in the El Paso plot (Table 1). Terpinolene yield was highest in the College Station plot (0.12 mg/g, Table 1).

In contrast to the mg/ g DM data, ANOVA of components on a % total oil basis showed a different pattern with four compounds being highly significantly different among plots (terpinolene, α -terpineol, (E)-caryophyllene, α -humulene) and two significantly different (bornyl acetate, humulene epoxide II) (Table 1). The major oil component, α -pinene, was not significantly different, ranging from 78.50% (College Station) to 64.43% (Arkansas).

Height and caliper measurements were largest in the Florida plot and lowest in the Kansas plot (Table 1). Percent chlorosis was greatest in Iowa and Arkansas, but chlorosis not a problem (0.0) in other plots (Table 1). Die back was most apparent in College Station, Arkansas and Kansas (Table 1).

PCA (Principle Components Analysis) factoring of the correlation matrix resulted in six eigenroots before they began to asymptote. The six eigenroots accounted for 42.9, 18.3, 11.6, 9.4, 7.4 and 3.8% (93.4%) of the variance among individuals. Factor loadings (as percent variance accounted for) are shown in Table 2. Oil yield and most monoterpenes (α -pinene, myrcene, limonene, β -phellandrene) as well as germacrene D (a sesquiterpene) and bornyl acetate are highly loaded onto component 1 (Table 2). However, β -pinene was not associated with α -pinene, but lightly loaded onto several components (eigenvectors). Terpinolene and (E)-caryophyllene have over 50% of their variance on component 2.

Component 3 accounts for modest amounts of several variables, but in no case, does it explain more than 38.8% (eudesma-4(15),7-dien-1- β -ol) (Table 2). Winter low temperature was loaded on component 4 (53.4%) and die back was loaded onto component 6 (Table 2). As with component 3, no variable was strongly loaded onto component 5. Most of the growth and edaphic variables were not strongly associated with any component (Table 2).

Ordination of the 24 variables of the first three components (accounting for 72.8% of the variance) shows two major groups: oil yield, monoterpenes, and some edaphic variables (winLo, sumHi, pH, dieb); and sesquiterpenes plus ppt, %chl, hgth and calip (Fig. 1). EU47 is isolated as is the CROX-HEII group. As seen in the factor loadings, growth variables (hgth, calip) are not closely ordinated with any terpenes as in the case of winter and summer temperatures (sumHi, winLo, Fig. 1).

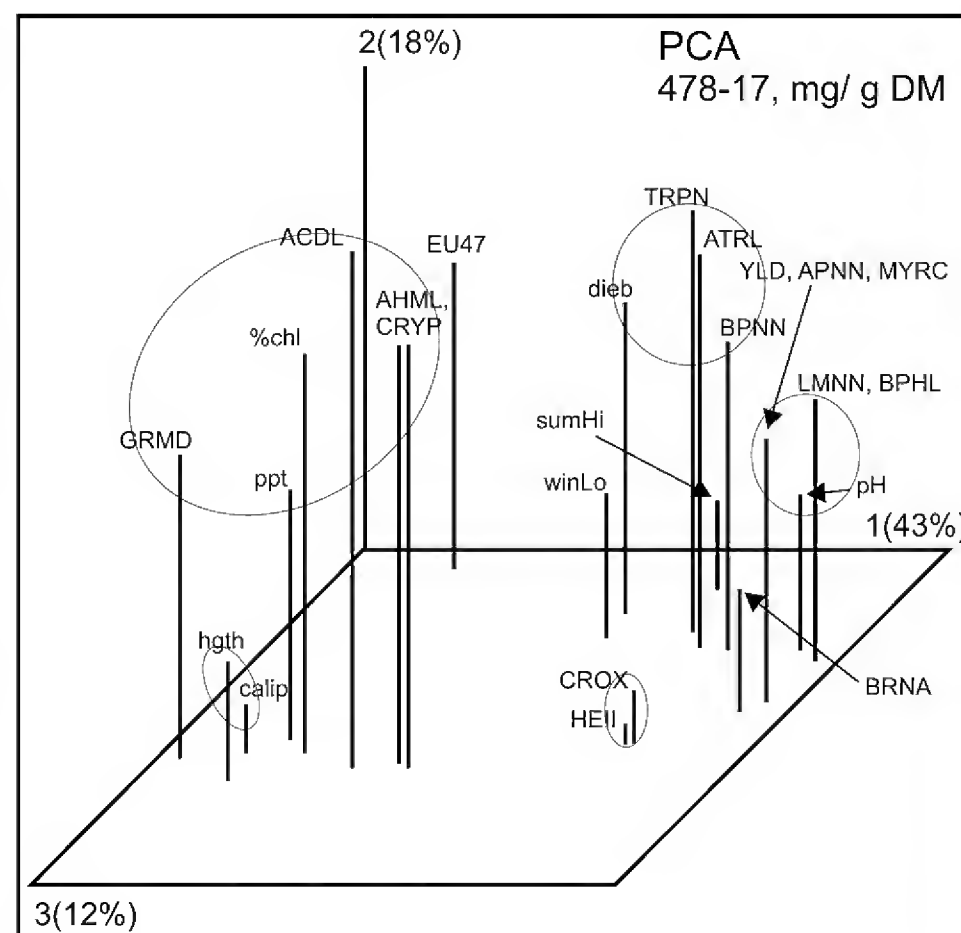


Figure 1. PCA of clone 478-17, mg/g DM basis. Circles show the major groups. See Table 1 for acronym abbreviations.

Clone 492-23

ANOVA of oil yield (mg/g DM) and 15 terpenoids for clone 492-23 revealed only one significant difference (eudesma-4(15),7-dien-1- β -ol) among the test plots, being highest in Arkansas and lowest in Iowa (Table 3). However, because most of the amounts of eudesma-4(15),7-dien-1- β -ol were very small (0.01 - 0.07), these statistics may be skewed due to the number of identical data values for this component. Oil yield was highest in the Kansas plot (7.27 mg/g) and decreased to the lowest level (4.60 mg/g) in Iowa (Table 3), but not significantly different.

As with 478-17, ANOVA of components on a % total oil basis displayed a different pattern than in the mg/ g DM data. Considerable variation was found with six compounds being highly significantly different among plots (α -pinene, β -pinene, (E)-caryophyllene, α -humulene, α -cadinol, eudesma-4(15),7-dien-1- β -ol) (Table 3) and one significantly different (germacrene D). The highest percent of α -pinene, the major component, was in highest Kansas (74.37%) and the lowest in Florida (59.40%).

PCA (Principle Components Analysis) factoring of the correlation matrix resulted in six eigenroots before they began to asymptote. The six eigenroots accounted for 39.4, 19.3, 14.4, 211.8, 5.4 and 5.2% (95.5%) of the variance among individuals (Table 4). Factor loadings (as percent variance accounted for) are shown in Table 4. Oil yield and all the monoterpenes (α -pinene, β -pinene, myrcene, limonene, β -

phellandrene, terpinolene) as well as α -terpineol were highly loaded onto component 1 (Table 4). Terpinolene and (E)-caryophyllene have over 60% of their variance onto component 2.

Germacrene D and α -cadinol (sesquiterpenes) were heavily loaded onto component 3 (Table 4). Height and caliper were heavily loaded on component 4 (75.5, 57.5%, Table 4). No single variable was associated with component 5, but 82.76% of the variance in die back was on component 6 (Table 4).

Ordination of the 24 variables of the first three components accounted for 73.1% of the variance. No major groups are apparent. Minor groups are: oil yield and monoterpenes; TRPN/ATRL; CROX/HEII; AHML/CRYP; and GRMD/ACDL. Growth and edaphic variables (winLo, sumHi, pH, dieb) do not seem to be highly correlated with individual terpenoids (Fig. 2).

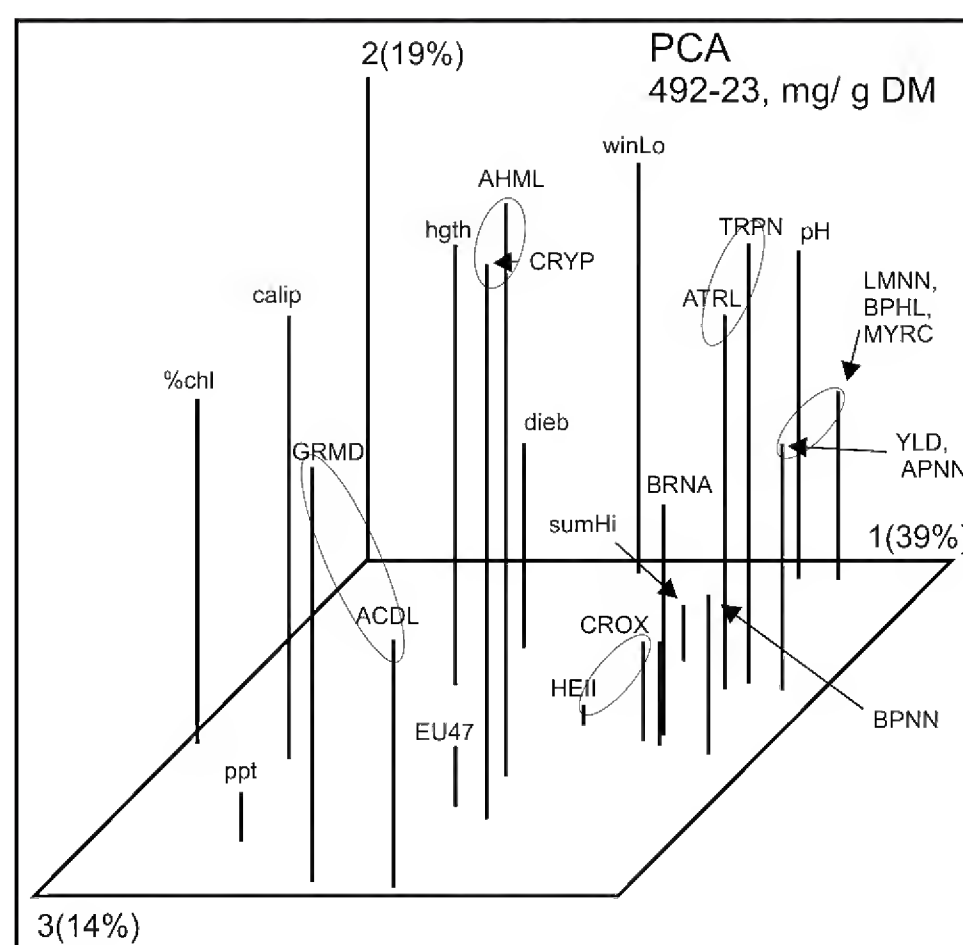


Figure 2. PCA of clone 492-23, mg/g DM basis. Circles show the major groups. See Table 2 for acronym abbreviations.

DISCUSSION

A surprising amount of variation was found in the leaf oils among clonal trees within a plot. This may be due to nature of foliage production and the large changes in terpene composition and yields in young (juvenile) and mature (adult) leaves in *Taxodium distichum*. Adams (2012), in a seasonal study, found the leaf oil increased rapidly in young leaves (April) to May (Fig. 3), then rapidly declined to July, became constant (statistically) until fall (Oct.). Three patterns of variation in the oil yield and terpenes were found: some compounds (eg. germacrene D, caryophyllene oxide, Fig. 3) increased during the summer, then declined in the fall; other compounds (caryophyllene, terpinolene, myrcene, Fig. 4) were highest in young leaves, then declined into the fall; and other terpenoid acetates and aldehyde (trans-pinocarvyl acetate, cis-pinocarvyl acetate, and germacra-4(15),5,10(14)-trien-1-al, Fig. 5) were absent

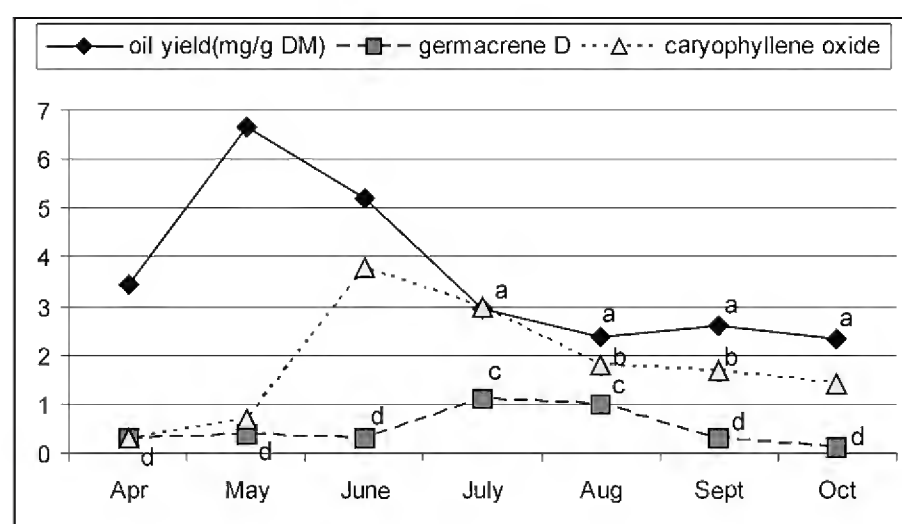


Fig. 3. Changes in oil yield, germacrene D and caryophyllene oxide during growth. (from Adams 2012).

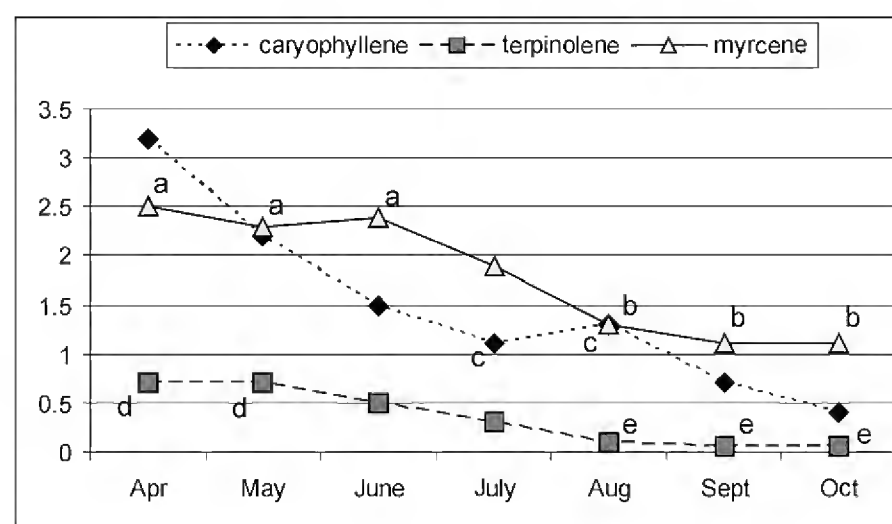


Fig. 4. Changes in caryophyllene, terpinolene, and myrcene during growth. (from Adams, 2012).

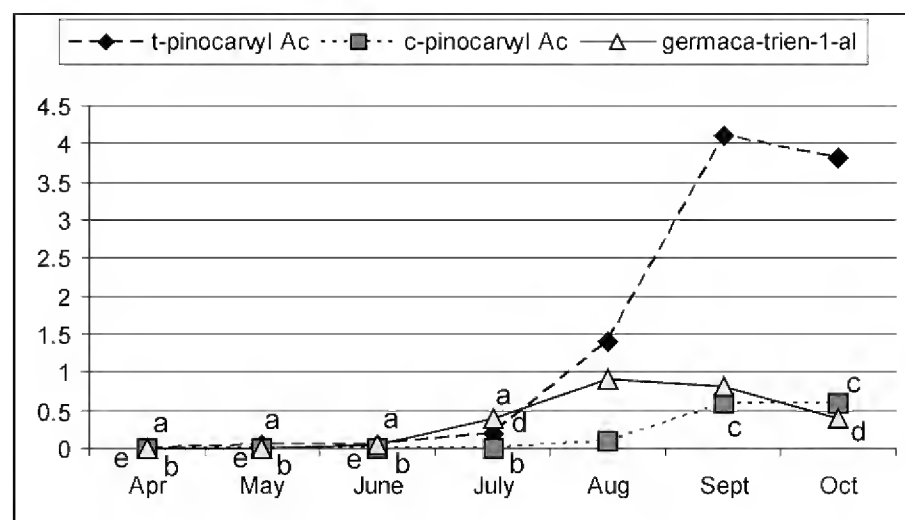


Figure 5. Changes in trans-pinocarvyl acetate, cis-pinocarvyl acetate, and germacra-trien-1-al during growth. (from Adams, 2012).

in young leaves and increased in the summer/ fall. Thus, it appears from that study, a major source of variation in oil yield and composition is differences in the ages of leaves. In the Adams (2012) study all foliage was collected from a single tree (genotype) and care was taken to remove any new flushes of leaves after the April sample (which was all young, juvenile leaves).

Thus, in the present study, even though foliage was collected on July 16 or 17, favorable growth conditions in the previous week or month, may have resulted in new flushes of foliage in some of our test plots. This likely led to a mixture of new and old leaves in the samples. This, in turn, could account for some of the variation found in the terpene composition and yields in this study.

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Table 1. Leaf essential oil composition (mg/ g DM and % total oil) for 478-17 from plots of *Taxodium distichum* in Iowa, Arkansas (Ark), Florida (FL), College Station, TX (Coll. Stat.), El Paso, TX and Kansas KS). Any number within a row with a common superscript is not significantly different ($P=0.05$ *). Significance = * (0.05); ** (0.01) ns = not significant; nt = significance not tested (variable too small). Variables with highly significant differences are in boldface.

	mg/ g DM data variable	El Paso, TX	College Station, TX	Kansas	Arkansas	Iowa	Florida	signif. P =
KI	oil yield (mg/g DM)	18.10 ^a	13.20 ^{ab}	10.65 ^{ab}	8.70 ^b	8.00 ^b	7.00 ^b	0.046 *
932	α -pinene	13.75 ^a	10.32 ^{ab}	8.34 ^{ab}	5.73 ^b	6.05 ^b	5.01 ^b	0.035 *
974	β -pinene	0.22	0.25	0.15	0.11	0.15	0.10	0.137 ns
988	myrcene	0.37	0.31	0.28	0.19	0.21	0.14	0.122 ns
1024	limonene	0.17	0.14	0.16	0.09	0.08	0.06	0.148 ns
1025	β -phellandrene	0.10	0.09	0.07	0.06	0.05	0.04	0.079 ns
1086	terpinolene	0.05 ^b	0.12 ^a	0.08 ^{ab}	0.06 ^{ab}	0.08 ^{ab}	0.04 ^b	0.031 *
1186	α -terpineol	0.08	0.19	0.11	0.15	0.10	0.08	0.010 ns
1287	bornyl acetate	0.19^a	0.10^b	0.09^b	0.11^b	0.06^b	0.06^b	0.010 **
1417	(E)-caryophyllene	0.16	0.22	0.20	0.20	0.30	0.20	0.439 ns
1452	α -humulene	0.02	0.03	0.02	0.03	0.04	0.03	0.557 ns
1480	germacrene D	0.00	0.00	0.00	0.01	0.02	0.01	nt
1582	caryophyllene oxide	0.88 ^a	0.35 ^b	0.28 ^b	0.54 ^{ab}	0.10 ^b	0.41 ^b	0.036 *
1608	humulene epoxide II	0.10 ^a	0.04 ^b	0.04 ^b	0.07 ^{ab}	0.01 ^b	0.05 ^b	0.020 *
1652	α -cadinol	0.00	0.01	0.01	0.01	0.01	0.01	nt
1687	eudesma-4(15),7-dien-1- β -ol	0.00	0.01	0.01	0.01	0.01	0.01	nt
	growth and site variables							
	height (cm)	106.5^b	125.0^b	91.5^b	135.7^b	170.0^b	388.0^a	0.002 **
	caliper (mm)	38.3^b	25.7^b	22.0^b	33.3^b	49.5^b	122.0^a	0.002 **
	% chlorosis	0.0^b	0.0^b	0.0^b	3.3^b	30.0^a	0.0^b	<0.001 **
	die back (cm?)	0.0	38.3	21.5	37.7	8.5	0.0	0.598 ns
	soil pH	8.4	7.1	6.0	5.6	5.2	5.3	
	winter low tempt. (F)	3.9	26.0	5.8	-17.0	-12.0	21.0	
	annual precipitate (in.)	8.0	19.4	28.2	53.8	32.5	44.3	
	summer high tempt. (F)	108.0	109.0	109.0	114.0	96.0	105.0	
	% total oil data variable	El Paso TX	College Station, TX	Kansas	Arkansas	Iowa	Florida	signif. P =
KI	% oil yield per g DM	1.81 ^a	1.32 ^{ab}	1.07 ^{ab}	0.87 ^b	0.80 ^b	0.70 ^b	0.046 *
932	α -pinene	76.10	78.50	78.15	64.43	76.75	69.80	0.325 ns
974	β -pinene	1.20	1.93	1.40	1.23	1.90	1.57	0.363 ns
988	myrcene	2.05	2.33	2.65	2.17	2.65	2.00	0.144 ns
1024	limonene	0.93	1.02	0.95	1.04	0.93	0.83	0.278 ns
1025	β -phellandrene	0.57	0.68	0.65	0.69	0.62	0.63	0.423 ns
1086	terpinolene	0.30^c	1.00^a	0.75^{ab}	0.70^b	1.00^a	0.63^b	0.001 **
1186	α-terpineol	0.45^c	1.43^{ab}	1.00^b	1.77^a	1.20^{ab}	1.17^{ab}	0.007 **
1287	bornyl acetate	1.05 ^{ab}	0.73 ^b	0.80 ^b	1.33 ^a	0.70 ^b	0.93 ^{ab}	0.046 *
1417	(E)-caryophyllene	0.80^d	1.63^c	1.95^{bc}	2.40^{bc}	3.75^a	2.80^{bc}	<0.001 **
1452	α-humulene	0.09^d	0.20^c	0.25^{bc}	0.33^{bc}	0.50^a	0.37^{bc}	<0.001 **
1480	germacrene D	0.00	0.00	0.02	0.02	0.20	0.04	nt
1582	caryophyllene oxide	4.75 ^{ab}	2.47 ^{ab}	2.65 ^{ab}	6.53 ^a	1.20 ^b	6.47 ^{ab}	0.060 ns
1608	humulene epoxide II	0.60 ^a	0.26 ^{ab}	0.35 ^{ab}	0.80 ^a	0.09 ^b	0.73 ^a	0.030 *
1652	α -cadinol	0.00	0.02	0.05	0.04	0.16	0.06	nt
1687	eudesma-4(15),7-dien-1- β -ol	0.00	0.01	0.03	0.02	0.02	0.05	nt

Table 2. Variance extracted by six eigenroots and percent variance explained by each variable onto eigenvector from PCA of 478-17 based on gm/g DM data for 16 terpene and 8 edaphic characters in 14 individuals in 6 test plots. Character codes are used in Fig. 1. Components (eigenvectors) accounting for 50% or more of the variance of a variable are in bold face.

		Component (eigenvector)					
		1	2	3	4	5	6
Variance by eigenvectors, % total:		42.9 %	18.3%	11.6%	9.4	7.4%	3.8%
code	mg/ g DM data set	Variance explained by each eigenvector (%)					
YLD	oil yield (mg/g DM)	91.7	0.9	5.5	0.2	0.0	0.3
APNN	α -pinene	87.2	2.6	3.1	2.1	0.1	1.4
BPNN	β -pinene	47.1	15.5	0.2	11.7	0.7	2.2
MYRC	myrcene	80.6	7.2	3.7	0.0	0.0	0.5
LMNN	limonene	77.8	2.5	0.1	0.0	0.1	0.1
BPHL	β -phellandrene	90.7	5.9	0.0	0.0	0.8	0.4
TRPN	terpinolene	27.9	53.8	3.2	0.8	10.5	0.5
ATRL	α -terpineol	21.0	28.1	8.6	11.6	19.9	5.5
BRNA	bornyl acetate	77.4	4.4	7.1	5.9	1.6	0.5
CRYP	(E)-caryophyllene	0.0	55.5	26.9	1.4	9.1	2.3
AHML	α -humulene	1.3	41.4	32.5	9.4	7.5	0.9
GRMD	germacrene D	58.3	11.6	22.4	0.1	2.2	0.7
CROX	caryophyllene oxide	40.7	27.5	12.7	11.1	1.2	2.9
HEII	humulene epoxide II	39.3	32.0	10.7	13.8	0.1	1.4
ACDL	α -cadinol	35.4	29.1	5.7	0.1	2.6	8.9
EU47	eudesma-4(15),7-dien-1- β -ol	33.6	9.6	38.8	0.2	0.7	4.7
hght	height (cm)	33.0	15.9	13.0	6.2	28.9	0.7
calip	caliper (mm)	24.5	21.8	20.0	7.5	20.5	0.9
%chl	% chlorosis	16.8	41.5	18.4	0.1	15.2	1.4
dieb	die back (cm?)	2.3	11.7	14.1	9.3	2.1	53.6
pH	soil pH	73.8	3.9	1.9	8.4	7.1	0.2
winLo	winter low tempt. (F)	4.5	1.7	5.5	53.4	32.5	0.1
ppt	annual precipitate (in.)	47.2	1.1	0.2	38.8	8.8	0.0
sumHi	summer high tempt. (F)	16.8	14.9	23.7	33.9	4.8	0.9

Table 3. Leaf essential oil composition (mg/ g DM and % total oil) for 492-23 from plots of *Taxodium distichum* in Iowa, Arkansas (Ark), Florida (FL), College Station, TX (Coll. Stat.), El Paso, TX and Kansas KS). Any number within a row with a common superscript is not significantly different (P=0.05 *). Variables with highly significant differences are in boldface.

	mg/g DM data variable	Kansas	College Station, TX	Arkansas	Florida	Iowa	signif. P =
KI	oil yield (mg/g DM)	7.27	6.87	6.10	5.45	4.60	0.250 ns
932	α -pinene	5.41	4.55	4.16	3.21	2.94	0.070 ns
974	β -pinene	0.13	0.12	0.14	0.09	0.10	0.342 ns
988	myrcene	0.25	0.23	0.18	0.16	0.15	0.077 ns
1024	limonene	0.07	0.07	0.06	0.05	0.04	0.070 ns
1025	β -phellandrene	0.05	0.05	0.04	0.04	0.03	0.218 ns
1086	terpinolene	0.05	0.06	0.03	0.04	0.03	0.329 ns
1186	α -terpineol	0.07	0.08	0.06	0.06	0.04	0.356 ns
1287	bornyl acetate	0.02	0.03	0.02	0.02	0.02	0.618 ns
1417	(E)-caryophyllene	0.39	0.39	0.23	0.47	0.43	0.319 ns
1452	α -humulene	0.05	0.05	0.03	0.06	0.05	0.276 ns
1480	germacrene D	0.05	0.03	0.06	0.16	0.09	0.263 ns
1582	caryophyllene oxide	0.16	0.24	0.26	0.14	0.06	0.166 ns
1608	humulene epoxide II	0.01	0.03	0.04	0.01	0.01	0.088 ns
1652	α -cadinol	0.04	0.04	0.08	0.10	0.04	0.172 ns
1687	eudesma-4(15),7-dien-1- β -ol	0.03 ^a	0.03 ^{ab}	0.07 ^{ab}	0.05 ^{ab}	0.01 ^{ab}	0.026 *
	growth and site variables						
	height (cm)	183.0^c	244.4^b	139.3^c	309.5^a	138.3^c	<0.001 **
	caliper (mm)	32.7^b	54.1^b	30.0^b	103.0^a	46.3^b	<0.001 **
	% chlorosis	0.0^b	0.0^b	3.3^b	0.0^b	36.7^a	<0.001 **
	die back (cm?)	0.0	38.3	37.7	0.0	1.7	0.370 ns
	soil pH	6.0	7.1	5.6	5.3	5.2	
	winter low tempt. (F)	5.8	26.0	-17.0	21.0	-12.0	
	annual precipitate (in.)	28.2	19.4	53.8	44.3	32.5	
	summer high tempt. (F)	109.0	109.0	114.0	105.0	96.0	
	% total oil data variable	Kansas	College Station, TX	Arkansas	Florida	Iowa	signif. P =
KI	% oil yield per g DM)	0.73	0.69	0.61	0.55	0.46	0.250 ns
932	α-pinene	74.37^a	67.63^{ab}	69.23^{ab}	59.40^c	63.77^{bc}	0.007 **
974	β-pinene	1.80^b	1.87^b	2.23^a	1.70^b	2.20^a	0.001 **
988	myrcene	3.43 ^a	3.40 ^a	3.00 ^{ab}	2.80 ^b	3.23 ^{ab}	0.056 ns
1024	limonene	0.99	1.03	0.95	0.93	0.94	0.265 ns
1025	β -phellandrene	0.68	0.70	0.65	0.62	0.63	0.220 ns
1086	terpinolene	0.67	0.83	0.53	0.60	0.77	0.235 ns
1186	α -terpineol	1.03	1.20	1.00	1.05	0.83	0.541 ns
1287	bornyl acetate	0.30	0.43	0.40	0.35	0.40	0.142 ns
1417	(E)-caryophyllene	5.37^{bc}	5.90^b	3.97^c	8.25^a	9.37^a	<0.001 **
1452	α-humulene	0.73^b	0.80^b	0.50^b	1.05^a	1.13^a	0.002 **
1480	germacrene D	0.73 ^b	0.53 ^b	1.00 ^b	2.35 ^a	1.83 ^{ab}	0.020 *
1582	caryophyllene oxide	2.13	3.73	4.07	2.70	1.33	0.171 ns
1608	humulene epoxide II	0.21	0.43	0.63	0.35	0.09	0.063 ns
1652	α-cadinol	0.57^b	0.53^b	1.23^a	1.65^a	0.77^b	0.006 **
1687	eudesma-4(15),7-dien-1-β-ol	0.36^b	0.37^b	1.13^a	0.90^a	0.20^b	0.001 **

Table 4. Variance extracted by eigenroots and percent variance explained by each variable onto eigenvector from PCA of 492-23 based on gm/g DM data for 16 terpene and 8 edaphic characters in 14 individuals in 5 test plots. Character codes are used in Fig. 2. Components (eigenvectors) accounting for 50% or more of the variance of a variable are in bold face.

		Component (eigenvector)					
		1	2	3	4	5	6
Variance by eigenvectors, % total:		39.4 %	19.3%	14.4%	11.8	5.4%	5.2%
code	mg/ g DM data set	Variance explained by each eigenvector (%)					
YLD	oil yield (mg/g DM)	91.8	0.4	1.1	0.8	0.1	1.3
APNN	α -pinene	80.4	0.1	0.5	3.5	6.2	4.2
BPNN	β -pinene	71.3	9.4	4.9	9.7	0.2	1.0
MYRC	myrcene	87.1	2.2	1.9	4.5	0.5	1.5
LMNN	limonene	92.4	0.0	1.0	0.9	0.1	1.2
BPHL	β -phellandrene	94.7	0.2	0.0	1.2	0.2	2.0
TRPN	terpinolene	63.7	17.1	0.6	4.2	0.0	2.3
ATRL	α -terpineol	69.3	5.2	0.0	0.1	8.8	0.2
BRNA	bornyl acetate	49.1	0.9	4.0	1.1	28.4	9.8
CRYP	(E)-caryophyllene	8.4	62.8	20.1	2.0	1.7	2.1
AHML	α -humulene	13.7	65.1	11.8	0.1	0.2	2.4
GRMD	germacrene D	1.0	16.6	77.5	0.0	0.3	3.2
CROX	caryophyllene oxide	32.6	32.4	0.0	9.1	22.43	0.0
HEII	humulene epoxide II	19.5	52.0	0.4	3.9	21.9	0.2
ACDL	α -cadinol	13.1	0.9	78.1	5.7	0.2	0.6
EU47	eudesma-4(15),7-dien-1- β -ol	17.4	38.9	30.7	8.3	0.0	0.5
hght	height (cm)	0.8	16.8	0.4	75.5	0.0	3.2
calip	caliper (mm)	8.4	22.5	6.7	57.5	0.1	1.1
%chl	% chlorosis	38.0	3.5	2.5	36.8	11.0	0.4
dieb	die back (cm?)	0.3	3.7	6.9	0.8	2.7	82.76
pH	soil pH	40.2	2.0	46.2	3.3	1.6	2.6
winLo	winter low tempt. (F)	14.8	32.9	9.1	40.2	1.6	0.0
ppt	annual precipitate (in.)	6.8	42.0	37.9	1.5	8.9	0.5
sumHi	summer high tempt. (F)	31.2	34.6	3.4	12.7	13.1	1.8

A new species of *Mikania* (Asteraceae: Eupatorieae) from south-central Oaxaca, Mexico

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ABSTRACT

Mikania pascualii B.L. Turner, **sp. nov.** is described from south-central Oaxaca. It is closely related to the rarely collected *M. globosa* Coulter (containing but a single floret per involucre, much as the latter), a taxon of Chiapas and Guatemala. A photograph of the type is provided, along with a map showing their distribution. Published on-line **www.phytologia.org** *Phytologia* 96(3): 178-180 (July 1, 2014). ISSN 030319430

KEY WORDS: Asteraceae, Eupatorieae, *Mikania*, Mexico, Oaxaca

Resembling ***M. globosa*** but having smaller leaves with shorter petioles; a narrower (1-4 cm wide vs ca 5 cm), more leafy capitulescence; heads arranged in smaller glomerules (ca 8 mm across vs 10-15 mm); and the involucre bracts narrower with rounded apices (vs truncate to emarginate).

Shrubs to 3 m high. **Stems** (upper) at first minutely pubescent but soon glabrate. **Leaves** (larger) alternate, 6-14 cm long, 3-8 cm wide; petioles 1.5-3.0 cm long; blades narrowly to broadly ovate, 3-nervate from near the base, glandular-punctate beneath, glabrous above and below, or nearly so (minutely pubescent beneath along the venation); margins entire their apices acute. **Capitulescence** a somewhat leafy, spike-like arrangement of rounded globules 8-15 cm long, 1-4 cm wide, the globules ca 8 mm in diameter, the latter composed of numerous small sessile heads, each with a single floret. **Involucres** ca 1.5 mm long, 0.5 mm wide, sparsely pubescent, especially along their margins; bracts 3-5 nervate, their apices rounded. **Corollas** narrowly funneliform, glabrous, ca 2 mm long; lobes ca 0.25 mm long and as wide. **Achenes** (immature) ca 1 mm long, sparsely hispid; pappus of ca 20 ciliate bristles ca 2 mm long.

TYPE: MEXICO. OAXACA: Distrito Pochutla; Mpio San Miguel del Puerto. “Camino a la Constanica, paraje la Union, cafetal del Faro. “Selva mediana subperennifolia con café.” 15.59 19.3 N, 96.07 23.1 W, 1282 m, 3 Mar 2013, *Jose Pascual* 2457 (Holotype: TEX).

According to label data the plant is an “Arbusto” 3 m high, although most authors have described the taxon as being a semiwoody vine. In the treatment of *Mikania* for Mexico by Holmes (1990), the novelty will key to *M. globosa*, a species of Chiapas and adjacent Guatemala (Fig.2). It differs from the latter in numerous characters, as noted in the above diagnosis.

The species is named for its collector, Jose Pascual Cortes (born 1956), “Don Chepe,” caretaker, guide and discriminating collector of plants of the coffee-growing zone where the type was collected, this suggested by Emily Lott, who supplied the data given here.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Holmes, V.C. 1990. The Genus *Mikania* (Compositae-Eupatorieae) in Mexico. *Sida Bot. Misc.* 5: 1-45.



Fig. 1. *Mikania pascualii* (Holotype: TEX).

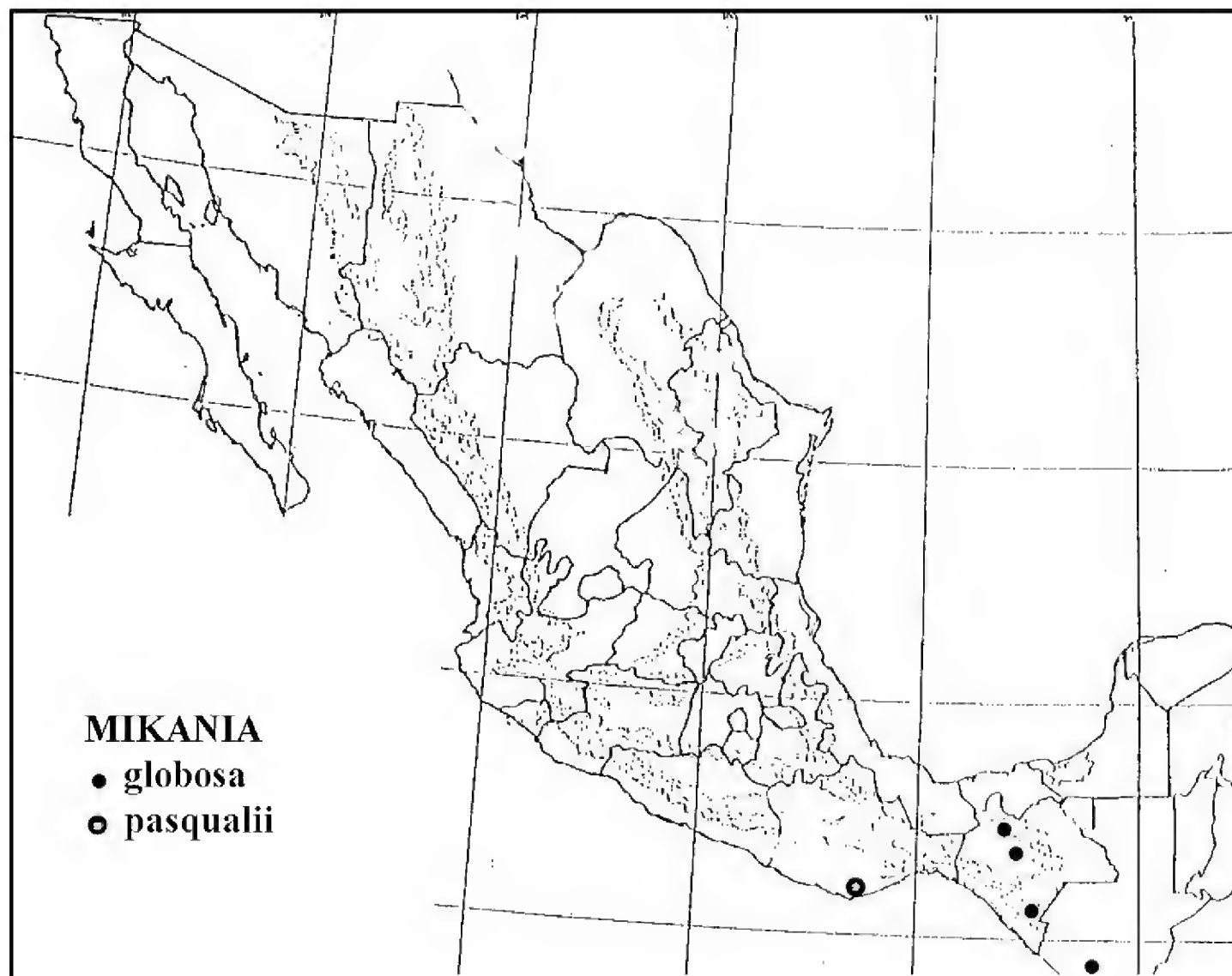


Fig. 2. Distribution of *Mikania pasqualii* and *M. globosa*.

Germination of seeds of *Streptanthus bracteatus* A. Gray, Bracted Twistflower (Brassicaceae), a Rare Central Texas Endemic

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ABSTRACT

Streptanthus bracteatus A. Gray, Bracted twistflower (Brassicaceae), is a rare central Texas endemic that appears to produce dormant seed; consequentially, acquiring seedlings for field and greenhouse studies is difficult. Factors promoting germination, including light levels and time to germination, were examined in the current studies. Germination of *S. bracteatus* seeds was significantly greater in the low light treatment than in the high light treatment (non-parametric Wilcoxon rank-sum test, $P = 0.0173$). Percent germination in the low light was 45% with 30/66 germinating. Germination in high light was 20% with 13/66 germinating. Almost all germinations occurred 5-9 days after seeds were wet. Based on seed mass, all seeds could have germinated; however, 67% of the *S. bracteatus* seeds were dormant. There may be another factor or combination of factors besides light level that could promote additional seed germination for this species. Published on-line www.phytologia.org *Phytologia* 96(3): 181-188 (July 1, 2014). ISSN 030319430.

KEY WORDS: seed dormancy, rare species, endemic, central Texas, Bracted twistflower, high light, low light

Many factors can affect the germination of seeds of flowering plants (Begon et al. 2006; Van Auken 2013). Seeds that fail to germinate when placed in conditions that normally promote growth are broadly termed dormant (Bradbeer 1988). There are various factors or conditions that can lead to dormancy, including immaturity of seeds, unfavorable growth conditions, and physical, morphological, or chemical barriers (Villiers 1972; Baskin and Baskin 2001). However, seed dormancy appears to be an important adaptation to help preserve an adequate number of viable seeds in the soil seed bank (Leck et al. 1989; Harlan 1992; Evans et al. 1996; Veasey et al. 2004; Finkelstein et al. 2008). Dormancy is especially important in buffering natural populations from extreme environmental conditions which can have major negative effects on seedling survival (Harlan 1992; Veasey et al. 2004).

Streptanthus bracteatus A. Gray, Bracted twistflower (Brassicaceae), is a rare herbaceous annual endemic to the Edwards Plateau ecoregion of central Texas. The recent and historic range of *S. bracteatus* is thought to include Bandera, Bexar, Comal, Hays, Medina, Travis, Real and Uvalde counties in south central Texas (Fig. 1) (Leonard and Van Auken 2013). Plants have been found in a wide range of habitats including dense live oak (*Quercus fusiformis*)/Ashe juniper (*Juniperus ashei*) woodlands, savannas, and intercanopy grassland gaps or patches (Van Auken 2000; Van Auken and McKinley 2008; Leonard and Van Auken 2013). Seeds germinate in early - late fall and can continue to germinate through much of the winter (personal observation) when temperatures become moderate and soil is wet by fall showers and soil moisture becomes non-limiting (Baskin et al. 1993). Plants will over-winter as basal rosettes and bolt in March or April to form a flowering spike (Fig. 2). Lavender flowers appear late in spring, usually from April to May (Correll and Johnston 1979). Fruits are siliques (Fig. 3) and can measure up to 15 cm in length. Siliques mature from late May to July and then dehisce, releasing the seeds (Correll and Johnston

1979). Most reports of germination of *S. bracteatus* seed are anecdotal, but germination of seeds from greenhouse-grown plants seem to be high (Zippin 1997). Seed collected from plants in the field appear dormant, thus making seedlings for ecological field and greenhouse studies difficult to obtain.

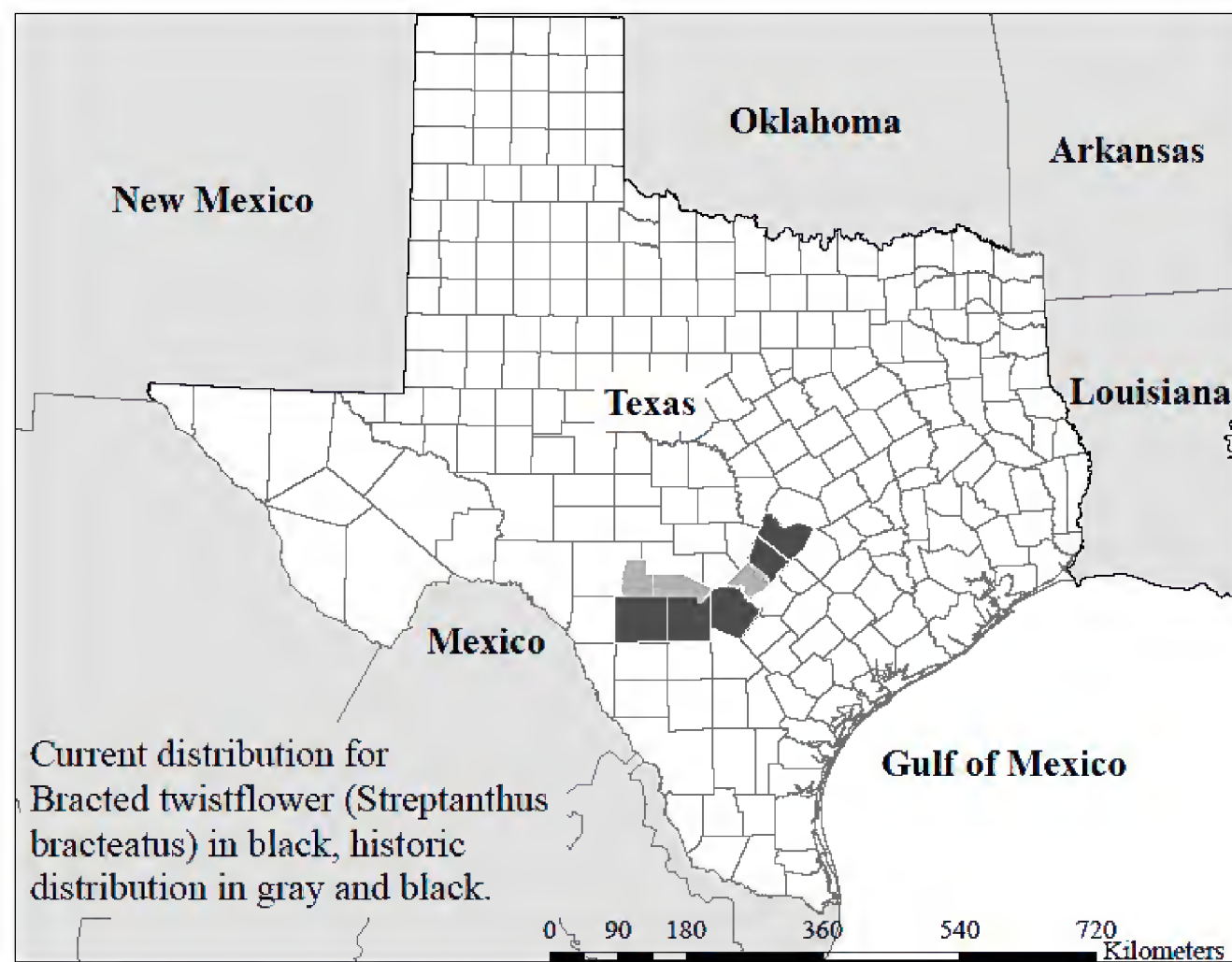


Figure 1. Distribution map for the Bracted twistflower (*Streptanthus bracteatus*). Current distribution in black and previous distribution in gray and black.

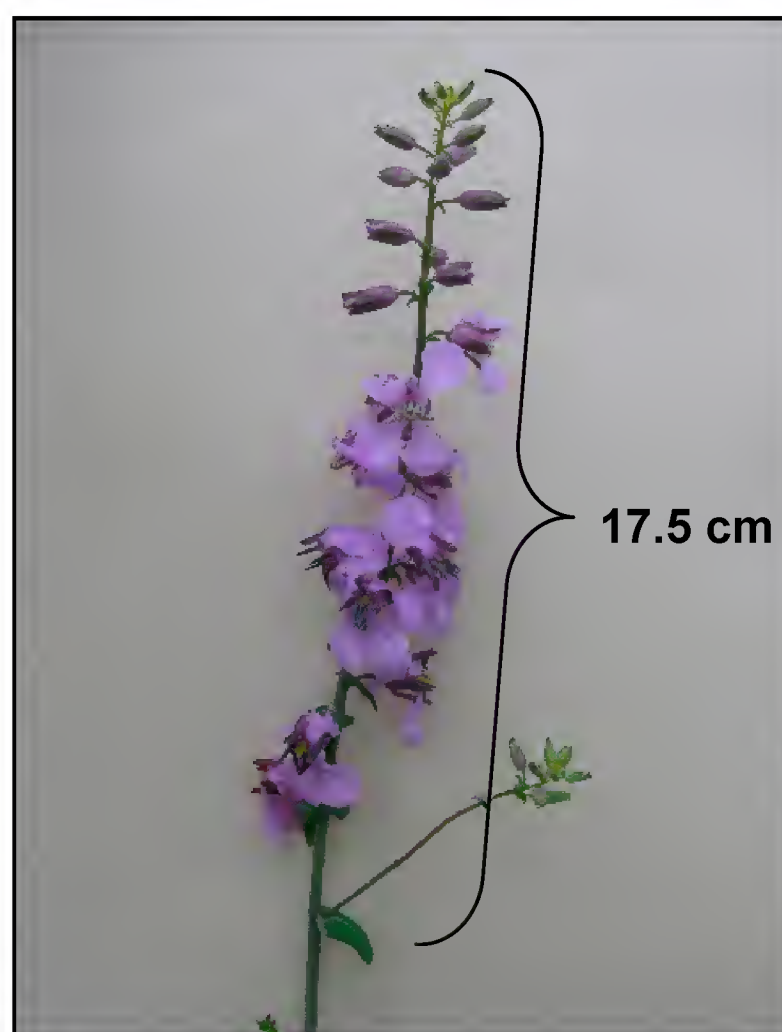


Figure 2. Picture of *Streptanthus bracteatus* inflorescence spike. Plants bloom in April and May in south Texas (Picture taken by senior author, April 2010).



Figure 3. Silique of *S. bracteatus* (9.4 cm long) containing up to 62 flattened seeds (Picture by senior author June 2010).

Simple germination tests can be conducted on seeds of any species and should be started soon after they are collected and then placed in a light-temperature regime that mimics or brackets natural habitat conditions for that species (Baskin and Baskin 2001). However, sometimes this is difficult to do, especially with rare species where plants, fruits and seeds may be uncommon and frequently hard to find. Various factors can be used to break dormancy and promote germination, including modified light levels, chilling, warming, or chemical exposure (Bradbeer 1988; Baskin and Baskin 2001).

Light level is very important in inducing germination in many species (McMillan 1986; McMillan 1987; Orth et al. 2000), as is temperature (Baskin et al. 1993). Photoperiod and specific wavelengths of light can also initiate germination of certain species (Orth et al. 2000). In non-dormant seeds, neither light nor darkness increases germination (Baskin and Baskin 1988; Baskin and Baskin 2001). In dormant seeds, germination is usually higher in high light, rather than in low light or dark treatments (Grime et al. 1981; Baskin and Baskin 1988; Baskin and Baskin 2001).

Germination is promoted for many species with a cold treatment or stratification by placing seeds on a moist substrate for approximately 12 weeks at 5°C (Baskin and Baskin 2001). It is important to note the time of year when seeds normally germinate in the wild. If plants flower and produce seeds in the spring and germinate in the fall, they would probably have higher germination after being exposed to high temperature (Baskin and Baskin 2001). Temperature regimes associated with native populations may be difficult to simulate, thus making dormancy difficult to break (Graae et al. 2008).

Our hypothesis was that *S. bracteatus* would have greater germination if seeds were grown under light levels typical of the environment where they are found. The goal of this study was to determine if germination of *S. bracteatus* seeds was improved by exposing them to increased light treatments.

MATERIALS AND METHODS

Two germination studies were conducted in 2013; results were similar and combined. Seeds were obtained from the Lady Bird Johnson Wildflower Center and were collected in July 2004 from wild populations in Medina County. The seeds were placed in airtight packaging and frozen at -18°C in

September of 2006, acting as a possible cold stratification. In October of 2008, seeds were removed from the freezer and thawed. Seeds remained in the airtight packaging at 4°C. On 24 January and 11 February 2013, six *S. bracteatus* seeds were evenly spaced in twenty-two, 100 mm diameter, polystyrene Petri dishes for a total of 132 seeds. Each Petri dish was filled with Scott's® Miracle-Gro Seed Starting Potting Mix® (approximately 8 g) that was moistened with deionized water at the start of the experiment. Subsequently, the soil and seeds were misted with deionized water as needed to maintain field capacity. Half of the Petri dishes were wrapped in aluminum foil to serve as a low light treatment and half of the dishes were left unwrapped to serve as the high light treatment (66 seeds in low light, 66 seeds in high light). Seeds in 16 Petri plates were started on 24 January and six on 11 February. Petri dishes were placed in 3.79 liter plastic bags to help retain moisture. All Petri dishes were placed 18 cm below a fluorescent grow-light on a metal rack. A timer was used to keep the grow-light on for 13 hours (from 6 am to 7 pm, CST). Temperature was measured using a stainless steel thermometer with a Vernier LabQuest® data logger. Light levels were measured with a FieldScout Quantum Light Meter® (Spectrum Technologies, Inc.). The average temperature was 22.0°C. The average light level was 46 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for the high light treatment and close to zero for the low light treatment.

Seeds in the Petri dishes were checked for germination for 15 days after wetting. Examination of the Petri plates for germination in the high light treatment was completed under the grow-light. Examination in the low light treatment was completed in a dark room with a dim, red head lamp. Any seeds that had cotyledons fully extended beyond the seed coat were removed and placed in 10 cm pots containing the same potting soil as above and transferred to a greenhouse located at Phil Hardberger Park, San Antonio, Texas for other experiments.

A non-parametric Wilcoxon rank-sum test was used to test the differences in the central tendencies of germination (%) for the light and dark treatments. Total number germinated for each survey date was also recorded. Total number of germinations per two days and the sum of the total number of germinations per two days were calculated and plotted.

RESULTS

Germination of the pooled results was significantly greater in the low light treatment than the high light treatment (Wilcoxon ranked sum test, $P = 0.0173$; Fig. 4). Number of germinations in the low light was 30/66 or 45%. For the high light treatment the number of seeds germinating was 13/66 or 20%

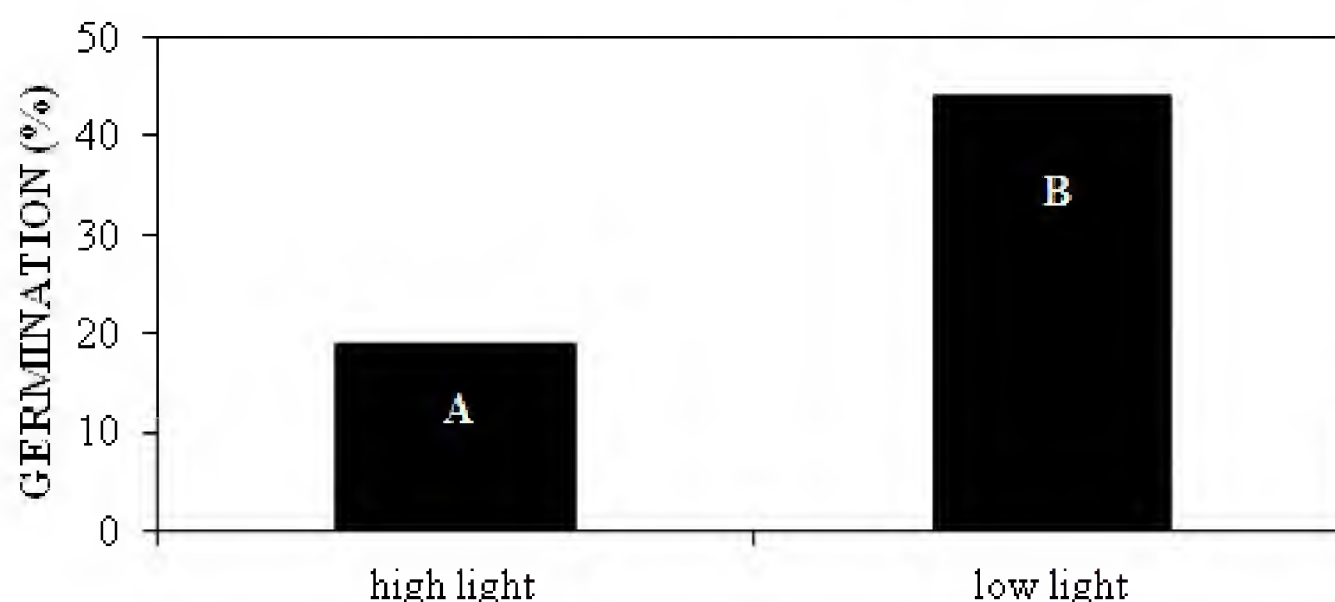


Figure 4. Percent germination for seeds of *Streptanthus bracteatus* in a germination study from January to February 2013 in high light and low light treatments. Different letters (A, B) within each bar indicate significant differences between the two light treatments (Wilcoxon ranked sum test, $P = 0.0173$).

(Fig. 4). Germinations occurred between the fourth and ninth day of treatment, peaking on day 7 (Fig. 5 and 6). After the ninth day, germination ceased for the low light treatment and was very low for the high light treatment. On the seventh day, both the low light and the high light treatment had the largest number of seeds germinating than any other day.

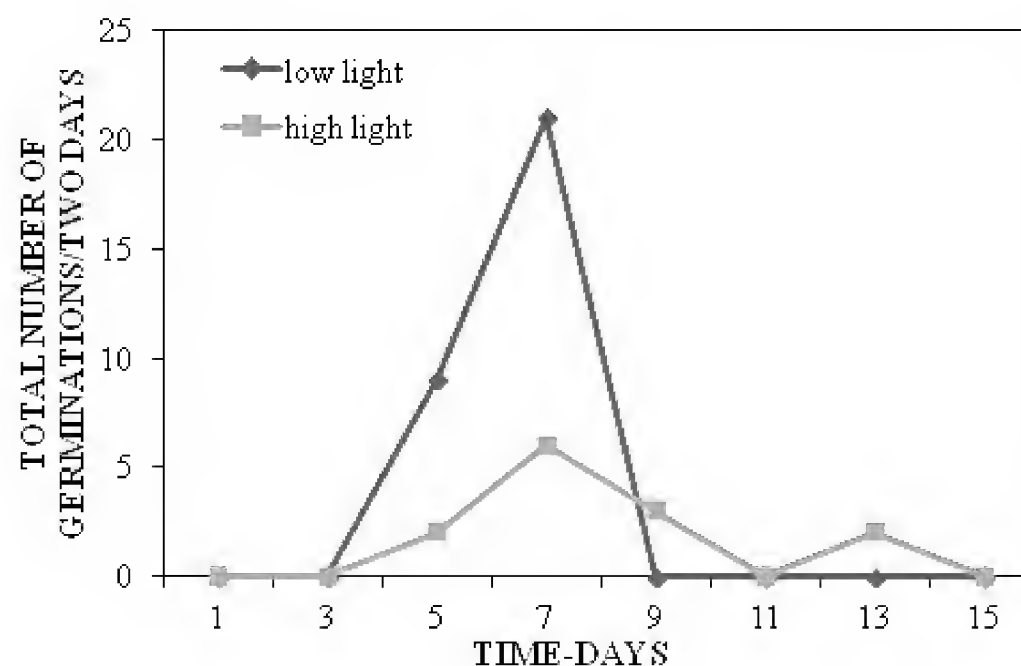


Figure 5. Number of germinations per two days for seeds of *Streptanthus bracteatus* in a germination study from January to February 2013 in low light and high light treatments.

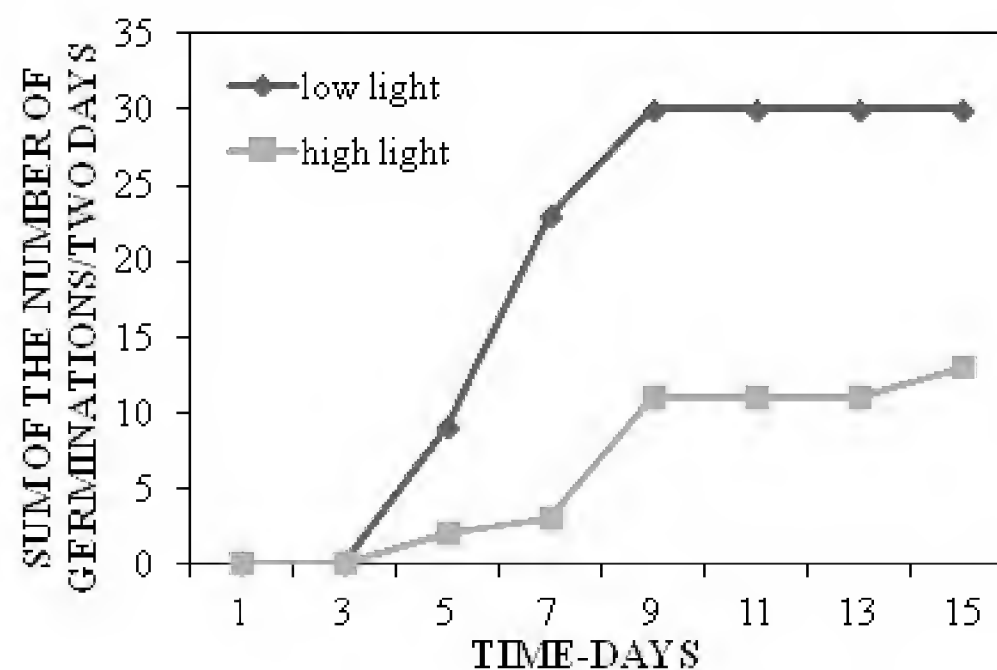


Figure 6. Sum of the total number of germinations per two day for seeds of *Streptanthus bracteatus* in a germination study at the University of San Antonio, San Antonio, Texas from January to February 2013 in low light versus high light treatments.

DISCUSSION

Most flowering plants exhibit some degree of seed dormancy (Begon et al. 2006). Mature seeds that fail to germinate even under favorable conditions require a dormancy-breaking agent to initiate germination (Villiers 1972). If dormancy occurs at the onset of seed release, then an environmental factor such as temperature or oxygen may be required for initiation of germination (Orth et al. 2000). Other dormancy-breaking factors may be necessary including hormones, photoperiod, water, nutrients, or mechanical scarification (Hilhorst and Karssen 1992; Gutterman 1994; Orth et al. 2000).

Streptanthus bracteatus seeds in the current study were frozen for over eight years before germination was attempted, which is not a standard cold treatment. Storage of seeds or achenes frozen in

a gene bank is a common preservation practice (Walters et al. 2005). Yet, the effect of long-term seed storage on most species, particularly rare native species, is generally unknown including effects on seed longevity and seed reserves. There seems to be considerable enthusiasm about collecting and preserving seeds of rare plants, but not for measuring initial viability or ability to germinate. Breaking dormancy or changing environmental controls preventing germination are mostly unknown effects of long-term, low temperature storage (Walters et al. 2005). If storage of the seeds at -18°C did cause dormancy-breaking, storage frozen for eight years probably allowed *S. bracteatus* seed after-ripening to occur. The seeds were probably physiological dormant and that dormancy was broken during low temperature dry storage.

Many species of the mustard family (Brassicaceae) have non-deep physiological dormancy and require after-ripening before germination will occur (Baskin and Baskin 2001). After-ripening can occur in stored seeds with non-deep physiological dormancy, regardless of how dormancy is broken in nature. In the soil or on the soil surface, mature seeds can after-ripen and have their dormancy broken by high summer temperatures or by low winter temperatures. Specifically, after-ripening will proceed faster at room temperatures compared to low temperatures, but low temperatures do not stop after-ripening entirely. If *S. bracteatus* seeds are not allowed to after-ripen (Baskin and Baskin 2001), essentially no germination will take place within 14 days of observation (personal observation).

A considerable amount of anecdotal information regarding high germination of *S. bracteatus* seeds is reported for greenhouse-grown plants (Zippin 1997). In the field, seeds of this species disperse in summer and start germinating in late September and early October, after they have been exposed to high summer temperatures (personal observation). We report 45% germination (Fig. 4) for our pooled experiments; seeds germinated better in low light than in high light. However, caution should be used when interpreting these results as the duration and intensity of light exposure could be important. *Streptanthus bracteatus* seeds were examined with a dim red lamp multiple times throughout the current germination experiment. Seeds germinated in darkness should not be exposed to any light until the end of the germination experiment (Baskin and Baskin 2001). The dim red light could have been enough to promote germination. *Streptanthus bracteatus* may require short durations of light to promote germination, but this is uncertain at this time. The germination for this species in this study was highest at 45% in low light.

Photoperiod and specific wavelengths of light can be important in initiating germination of certain species (Orth et al. 2000). However, in non-dormant seeds, neither light nor darkness increased germination (Baskin and Baskin 1988; Baskin and Baskin 2001). In dormant seeds, germination is usually higher in high light, rather than in low light or dark treatments (Grime et al. 1981; Baskin and Baskin 1988; Baskin and Baskin 2001). Only a few species germinate at higher levels in low light or dark rather than high light treatments (Baskin and Baskin 2001). Also, germination may be dependent upon the duration of light received; for example, long exposure rather than short exposures (Pons 2000). Long high light exposure inhibits mustard seed germination in some species (Pons 2000). Lettuce (Brassicaceae) seeds are negatively affected by long exposure to high light levels but requirements vary with temperature (Baskin and Baskin 2001; Gorski and Gorska 1979). More than 80% of *Lactuca sativa* seeds germinated in light when exposed to temperatures of $10\text{--}30^{\circ}\text{C}$; yet, when placed in darkness, germination was 0% at 30°C and >45% at temperatures of $10\text{--}22^{\circ}\text{C}$ (Evenari 1952; Baskin and Baskin 2001).

Continuous exposure to high irradiance may be inhibitory during the germination process (Baskin and Baskin 2001). Germination in three species of *Brassica* (mustard) was inhibited at the light levels of $0.5\text{ mol m}^{-2}\text{ day}^{-1}$ or higher (Baskin and Baskin 2001). Short-day seeds like annuals that germinate in the fall or winter can be inhibited by continuous light, whereas long-day seeds are not inhibited by continuous light (Evenari 1965; Baskin and Baskin 2001). *Streptanthus bracteatus* is a winter annual that typically germinates in the fall when day length begins to shorten.

The percent of *S. bracteatus* seeds not germinating in the present study may be ecologically important for the long-term survival of this species in a habitat that fluctuates dramatically in both temperature and rainfall (Van Auken and McKinley 2008).

ACKNOWLEDGEMENTS

We sincerely appreciate C. C. Baskin for helping us understand seed after-ripening and what can happen to seeds with cold, dry storage. We would also like to thank Minnette Marr, Paul Cox, and Ron Tullius for their review of the manuscript.

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**The closed flower of *Funastrum utahense* (Engelm.) Liede & Meve
(Apocynaceae: Asclepiadoideae)**

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ABSTRACT

A remarkable milkweed, *Funastrum utahense*, is shown by photos and diagrams to have replaced the corona by a highly-modified corolla. Published on-line www.phytologia.org *Phytologia* 96(3): 189-194 (July 1, 2014). ISSN 030319430

KEY WORDS *Funastrum utahense*, Apocynaceae, Asclepiadoideae, California, Nevada, Arizona, Utah.

This fairly small perennial vine with its unusual flowers ranges from southeastern CA, across southern NV, western AZ, to southwestern UT. It was first described by George Engelmann (Parry, 1875) as *Astephanus utahensis* from a specimen collected in the southwestern corner of UT. The genus name is Greek for ‘without a corona.’ There are at least four published drawings of the plant and its flower. A drawing of the flower alone was published in Jepson’s Manual (1927), but shows the flower with a campanulate, un-furrowed corolla. Jaeger (1940) published a small sketch of the plant and gave it the common name "Deboltia", but it is referred to as "Utah vine milkweed" in Jepson's Manual, 2nd ed., 2012. Woodson (1941) in his major reorganization of the North American Asclepiadaceae, transferred it to *Cynanchum*, where it remained as *C. utahense* for half a century. Even so, Janish published the best drawing I’ve seen of it in Abrams’ Flora (1951) but still under the name, *Astephanus utahensis*. Finally, Liede & Meve (2002) placed it in *Funastrum*. They included a drawing of the flower similar to my own (Fig. 2, D), but without discussion of the significance of the remarkable corolla. The present paper provides a closer view of this odd little flower.

OBSERVATIONS AND CONCLUSIONS

The flowers of *F. utahense* are much smaller than the other U.S. members of the genus. But what makes them so remarkable is that the open flowers are scarcely distinguishable from the closed ones, and they completely lack the fairly large, highly-visible coronas of the other members. According to Kunze (1991) they are “closed flowers,” i.e. flowers in which only the proboscis of the insect enters the flower, while its body remains outside of it. In his original description, Engelmann postulated that the nearly-closed and internally-glandular-papillose corolla took the place of the corona, the purpose of which is to position the insects for pollination. When the flowers are open, there is only a small hole in the apex of the corolla, and sometimes 5 narrow, horizontal slits between the corolla lobes, and these are so small that any insects able to enter the flowers would be too small to pollinate them. It would seem that the tiny openings in the *F. utahense* flowers are intended solely to admit a proboscis. Delpino (in Hildebrand, 1867) noted that in milkweed flowers that have the nectar reservoirs alternate to the anther wings, as in the hoods of *Asclepias*, the corpuscula become attached primarily to the leg bristles of insects, but in those species where the nectar reservoirs are positioned beneath the anther wings, the corpuscula become attached to the proboscises. It has long been known (Galil and Zeroni, 1965) that in *Asclepias* the nectar is secreted by the filaments just behind the anther wings, and travels by tiny ducts to the hoods. In *Funastrum*, which lacks hoods, the nectar would flow downward and pool in reservoirs directly below the anther wings (see Fig. 2, F). However, since in *F. utahense* the corpuscula are covered by the arched lobes of the corolla, when the insect begins probing the flower with its proboscis, it is guided to the nectar reservoirs and to the anther wings by the alternating furrows of the pentagonal corolla tube (see Fig. 2, E). Engelmann was correct; in *F. utahense* the corolla takes the place of the corona so completely that

nothing has been omitted. Moreover, the interior of the flower is so densely papillose that it has the appearance of being carpeted. I suspect that these papillae provide the scent that attracts the insects to the flowers, at least those with proboscises 4-5 mm long.

In the population I examined in eastern San Bernardino Co., CA, near the Providence Mts., fruits were fairly common in May, 2005. It had been a very wet fall and winter. The plants were growing in the barely-perceptible drainage channels that fanned out across the nearly flat plain. I never found them outside of these channels.

ACKNOWLEDGEMENTS

I express my appreciation to James M. Andre, whose photos and location data on the internet enabled me to locate this site and study these remarkable plants. I also express my gratitude to Thomas J. Rosatti and Sigrid Liede-Schumann for reviewing the manuscript, and to Victoria MacMichael and Roy Gereau for their assistance in preparing it.

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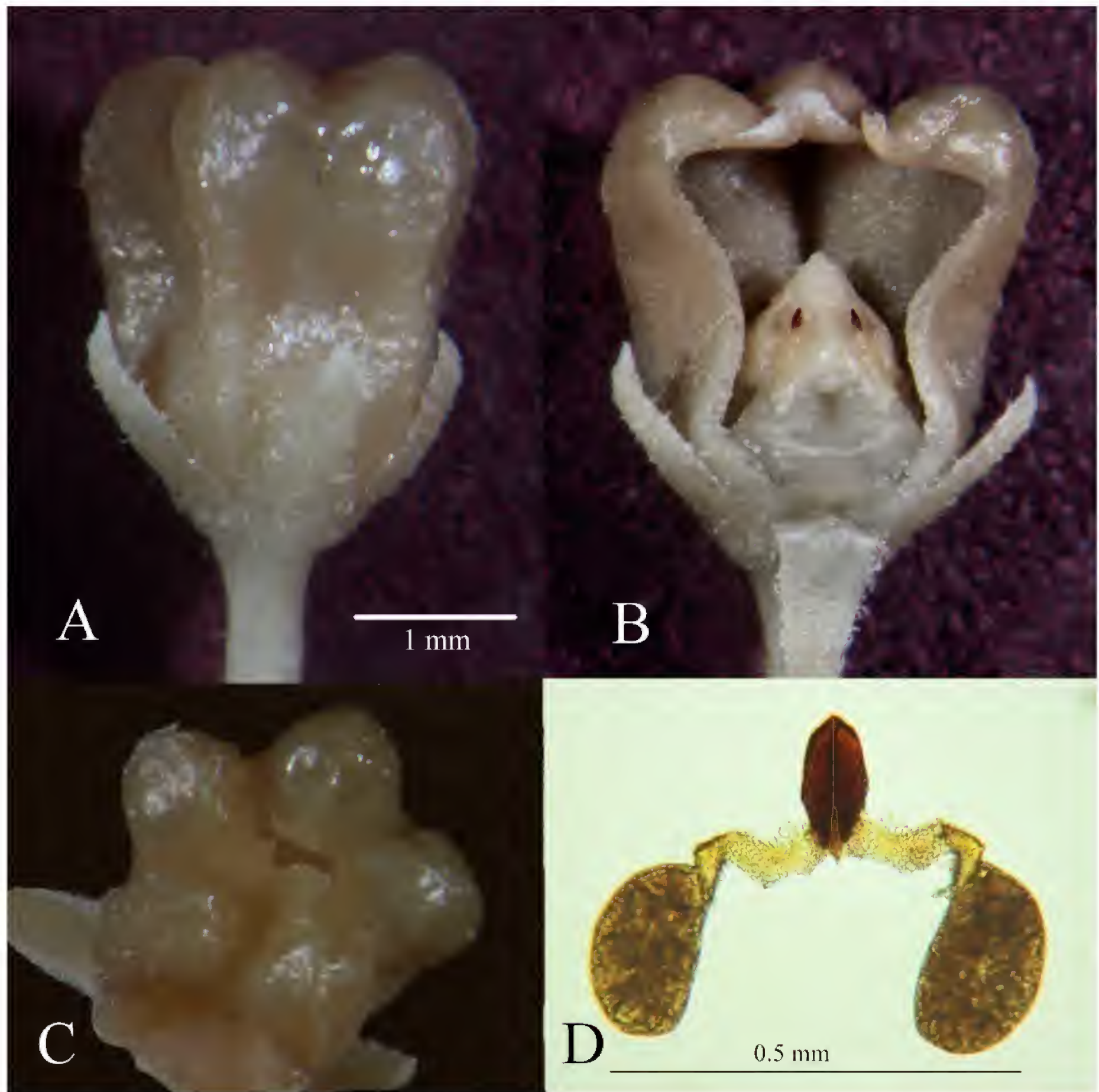


Figure 1. *Funastrum utahense* from flowers in alcohol. A. Intact flower in side view; the entire flower is about 4 mm long. B. Flower with two lobes cut away to show the interior; note the textured inner walls of the corolla and the deep furrow behind the gynostegium. C. Open flower from the top. D. Pollinarium.

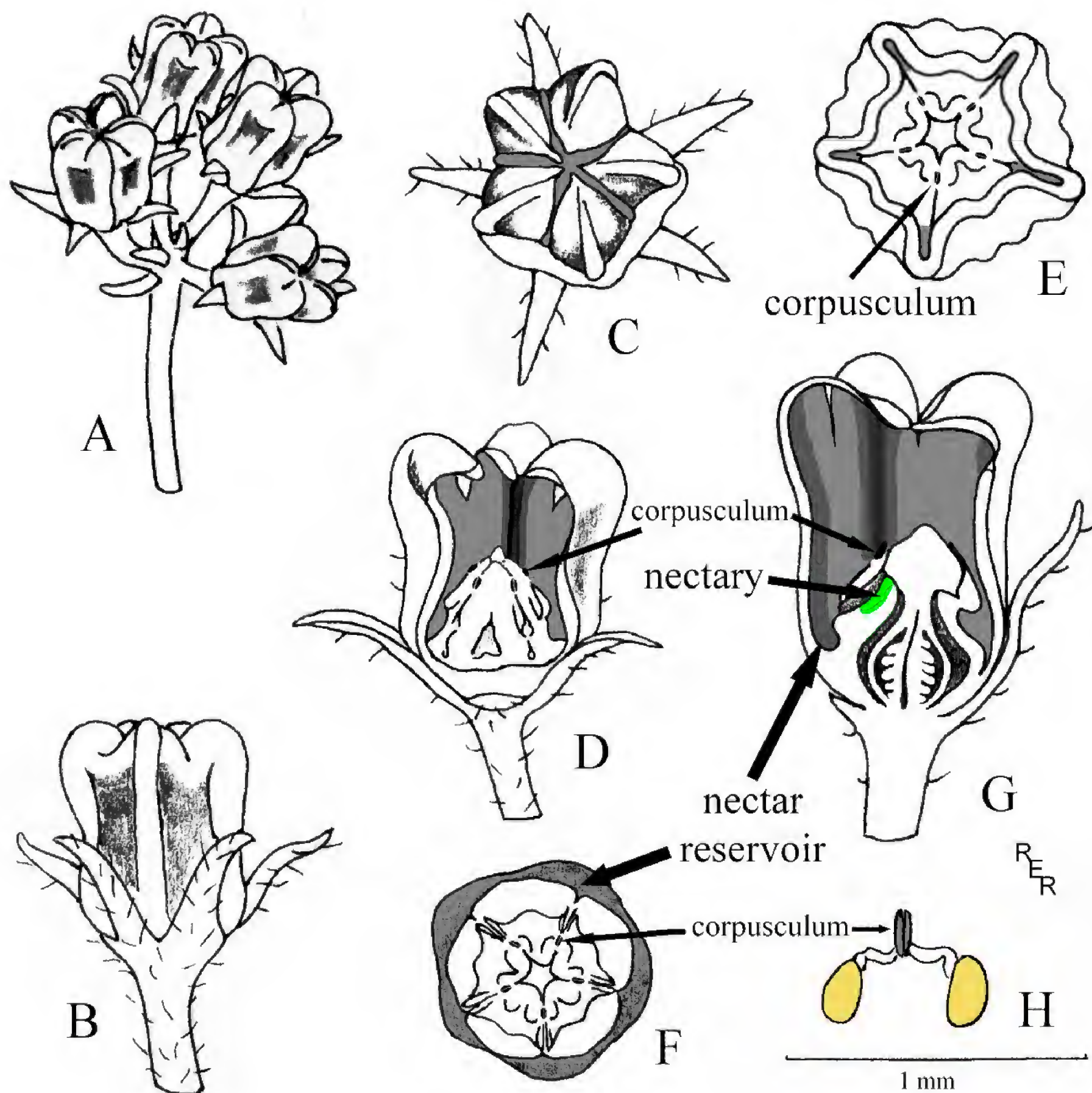


Figure 2. *Funastrum utahense*. A. Umbel of flowers. B. Flower in side view. C. Open flower from the top. D. Flower with two lobes removed to show the gynostegium. E. Flower with the top cut away to show the deeply-furrowed, pentagonal shape of the corolla. F. Gynostegium from the top showing the nectar reservoirs just below the anther wings. G. Flower in radial section. H. Pollinarium.



Figure 3. *Funastrum utahense*. Blooming plant. The flowers turn from yellow to orange as they age.



Figure 4. *Funastrum utahense*. Plant with three fruits.

The genus *Asanthus* (Asteraceae: Eupatorieae) revisited

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ABSTRACT

My previous publications relating to the genus *Asanthus* are reviewed and compared with the taxonomic findings of yet other workers, especially those having to do with DNA studies. It is concluded that *Asanthus* is composed of 3 species: *A. squamulosus*, *A. solidaginifolius* and *A. thrysiflorus*. It is noted that the latter two are sufficiently close so as to be considered as but varietally distinct, such designation awaiting additional study. A key to the several species is provided, along with maps showing their distributions. Published on-line www.phytologia.org *Phytologia* 96(3): 195-198 (July 1, 2014). ISSN 030319430

KEY WORDS: Asteraceae, Eupatorieae, *Asanthus*, *Brickellia*, *Steviopsis*, Mexico

King and H. Robinson (1972) erected the genus *Asanthus* to accommodate three taxa, these previously treated by B. L. Robinson (1917) as belonging to the section *Steviastrum* of *Brickellia* (*B. solidaginifolia* and *B. thrysiflora*) and the monotypic sect. *Gemmipedium* of *Brickellia* (*B. squamulosa*). All of these have glabrous non-bulbous stylar shafts, like those of *Steviopsis*, and all, except *B. squamulosa*, have similar subimbricate involucre bracts. McVaugh (1984) retained this complex in *Brickellia*, largely because of their 6-8 ribbed achenes (which I interpret as basically 5-ribbed with intercalary, smaller ribs, unlike the 8-9 equally-ribbed achenes of most *Brickellias*), all of which have swollen, pubescent, stylar shafts. King and H. Rob. (1987) provided an overview of the *Asanthus* complex in which 3 species were recognized: the very distinctive type species, *A. squamulosus*, and the two more similar taxa, *A. solidaginifolius* and *A. thrysiflorus*. Turner (1997), however, presumably in a lumping mood, positioned the several taxa in his broad concept of *Steviopsis*.

I now recant such a treatment, what with the recent DNA studies of Schilling et al. (2013) that confirm the taxonomy of King & Robinson (1987). In short, all of the above-mentioned taxa are best removed from *Brickellia* and/or *Steviopsis* and positioned in *Asanthus*, the latter readily distinguished from *Steviopsis* by having glandular (vs eglandular) achenes, and erect (vs spreading) corolla lobes. In addition to the taxonomy, the purpose of the present paper is to provide a key to the species, showing their distributions in greater detail than that provided by previous workers.

King and H. Robinson provided an excellent description of the genus, and such need not be replicated here.

Key to species

1. Leaves linear, 4-6 mm wide, mostly deciduous when achenes approach maturity; n Dur, Chi and USA.....**A. squamulosus**
1. Leaves narrowly lanceolate to ovate, 6-20 mm wide, persistent through maturity; north-central Mexico...(2)
2. Involucres 2-4 seriate; inner bracts 1 mm wide or less; n Dur, Chi, Nue.....**A. solidaginifolius**
2. Involucres 4-6 seriate; inner bracts 1-2 mm wide; south-central Mexico.....**A. thrysiflorus**

ASANTHUS SOLIDAGINIFOLIUS (A. Gray) King & H. Rob., Phytologia 24: 66. 1972.*Brickellia solidaginifolia* A. Grayn Dur, Chi and Nue; oak-pine woodlands, 1600-2600 m; Sep-Nov. **Map 1**

Suffruticose herbs to 1 m high. **Leaves** numerous and mostly alternate, 3-8 cm long, 0.4-0.8 cm wide; petioles 1-5 mm long; blades lanceolate, 3-nervate from or near the base, puberulent, prominently glandular-punctate or atomiferous glandular, the margins entire or nearly so. **Heads** numerous on terminal much-branched stems, forming an elongate, broad capitulescence, the ultimate peduncles 1-15 mm long. **Involucres** 2-4 seriate, 6-7 mm high, the bracts broad and 2-4 striate, or slender and 1-2 striate. **Florets** 10-14 per head, the corollas 6-7 mm long. **Achenes** 3.5-5.0 mm long, 9-ribbed, sparsely glandular pubescent, the pappus 1-seriate, composed of ca 30 barbellate or subplumose bristles 6-8 mm long.

Turner (1988) proposed this taxon as a variety of *A. thrysiflorus*, such never **formally** published, hence not listed in IPNI. See additional discussion below.

ASANTHUS SQUAMULOSUS (A. Gray) King & H. Rob., Phytologia 24: 66. 1972.*Brickellia squamulosus* A. Gray*Steviopsis squamulosa* (A. Gray) B.L. Turner

Chi, n Dur, Coa and USA, mostly in sandy soils along flood plains and arroyo bottoms, 2000-2200 m; Apr-Jun. **Map 2**

Suffruticose herbs or shrublets to 6 dm high; stems erect, brittle, corky and glabrate at maturity. **Leaves** subopposite to alternate, 3-7 cm long, 2-6 mm wide; petioles 0-2 mm long; blades (on primary growth) linear, 3-nervate from below, the margins entire or nearly so; leaves on secondary growth forming minute bead-like clusters that persist after the primary leaves drop. **Heads** numerous, turbinate, arranged in narrow, compact, elongate, wand-like capitulescences. **Involucres** 5-8 seriate, very graduate, 8-10 mm high, the phyllaries indurate, puberulent. **Florets** 8-12 per head, the corollas ca 8 mm long. **Achenes** 3-4 mm long, 10-ribbed, glabrous or nearly so, the pappus 2-3 seriate, of numerous nearly eciliate bristles, 6-8 mm long.

Asanthus squamulosus is superficially similar to *Brickellia spinosa*, as noted by Robinson (1917). King and Robinson (1972a), however, called to the fore the many differences between the two taxa especially the nodose stylar shafts, placing this in the genus *Asanthus*, while Turner (1997) placed the taxon in *Steviopsis*. DNA data (Schilling et al. 2013) confirm the classification of King and Robinson.

The latter workers provide an excellent full-page sketch of the species.

ASANTHUS THYSIFLORUS (A. Gray) King & H. Rob. Phytologia 24: 66. 1972.*Brickellia thysiflora* A. Gray*Steviopsis thysiflora* (A. Gray) B.L. Turner

Nue, Dur, Agu, Zac, San, Jal and Gua, oak-pine woodlands, 1600-2600 m; Sep-Nov.

Suffruticose perennial herbs to 1 m high. **Leaves** numerous and mostly alternate, 3-8 cm long, 4-8 mm wide; petioles 0-5 mm long; blades lanceolate, 3-nervate from or near the base, puberulent, prominently glandular-punctate or atomiferous-glandular, the margins entire or nearly so. **Heads** numerous on the much-branched stems, forming an elongate, broad, capitulescence, the ultimate peduncles 0-15 mm long. **Involucres** 4-6 seriate, 6-8 mm high, the bracts narrow and 1-2 striate. **Florets** 10-14 per head, the corollas 6-7 mm long. **Achenes** 3.5-5.0 mm long, 9-ribbed, sparsely pubescent, the pappus 1-seriate, of ca 30 barbellate or subplumose bristles 6-8 mm long.

As noted above, King & Robinson (1987) recognized 2 species within the *Asanthus thrysiflorus* complex, while McVaugh (1984) reduces these to synonymy under his *Brickellia thysiflora*. Turner (1988) treated the two taxa informally as varieties, largely because they were allopatric and some of the characters seemed to intergrade. McVaugh (1984) recognized the regional variation discussed above, but synonymized the species on the basis of presumed intermediates. King and H. Robinson (1972) and

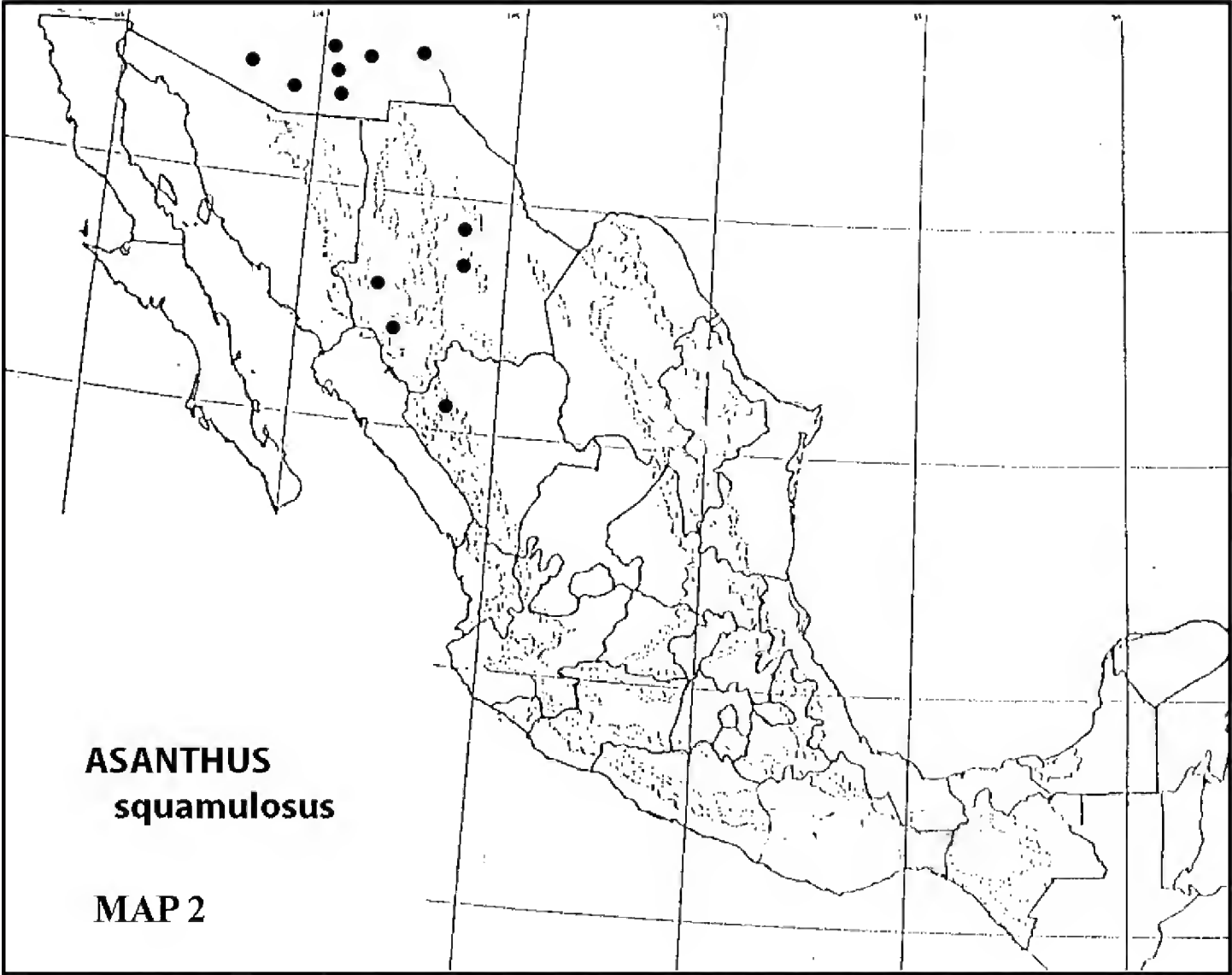
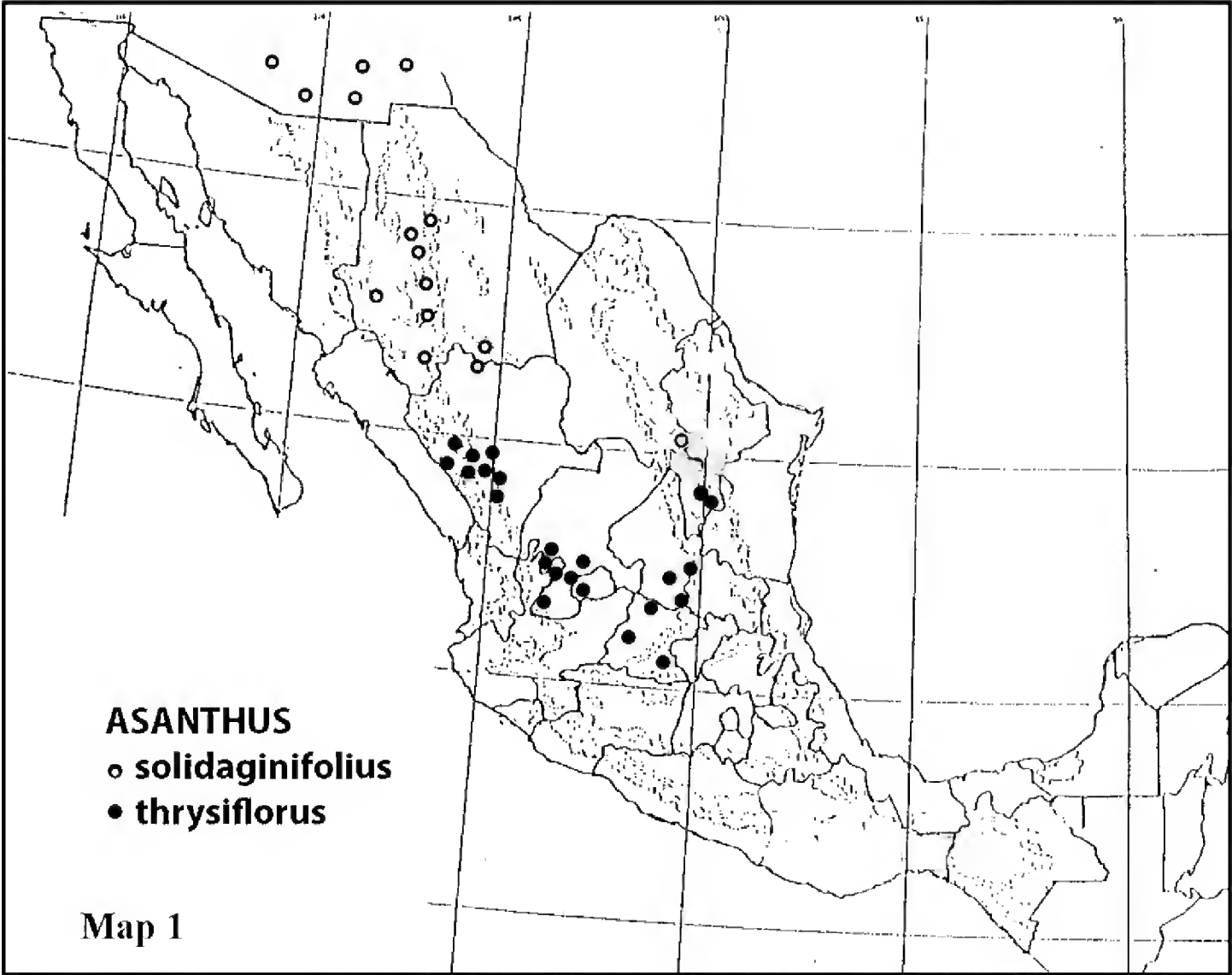
Schilling et al. (2013), treated these as species, but the latter workers noted "the decision to recognize them as distinct species or as varieties (or subspecies) within a species is still open." I have reexamined the complex and conclude that the proposed infraspecific taxa are worthy of recognition. I note further, Schilling et al.'s DNA citation 2491 (from Coa, *Villarreal* 5422, TEX) appears to be an immature specimen of *Asanthus solidaginifolius* (with which it clusters in their Fig. 2) and not *A. thrysiflorus*. Additionally, I should add that the two specimens from Nuevo Leon indicated in my Map 1 possess unusually large heads, their involucre (8-10 mm high); these perhaps represent an unnamed taxon, albeit varietal.

ACKNOWLEDGEMENTS

Thanks to my colleague, Tom Wendt for curatorial information. My editorial assistant, Jana Kos read the paper and provided helpful comments. The dot maps are based upon specimens on file at GH, LL-TEX and specimens cited in the literature that seem acceptable.

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***Ageratina jalpanserra* (Asteraceae: Eupatorieae), A new name for *A. jalpana* B.L. Turner (2014),
not *A. jalpana* B.L. Turner (1997)**

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In a recent paper (Turner 2014) I proposed a novelty, *Ageratina jalpana* B.L. Turner, from the Mpio. Jalpan de Serra, Queretaro, Mexico, noting:

The species is named for the Municipio de Jalpan, whence the type; not to be confused with **A. jalpana** B.L. Turner from Zacatecas, this named for the city of Jalapa (Turner 1996).

Unfortunately, I failed to note that a typo had been made in my 1997 paper, having failed to catch the misspelling of my intended jalapana as “jalpana.” Upon publication of my 2014 paper, my friend Harold Robinson of US almost immediately called attention to the oversight, followed soon thereafter by my colleague K. Gandhi of GH, who noted that the more recent novelty, under the Code of Nomenclature would require a new name.

I responded to Gandhi: “My first *Ageratina* jal[a]pana was a spelling error; I do not know how it happened; perhaps another reviewing editor omitted the “a” I called this to the attention of H. Robinson, as I noted in the email I just sent you. I purposely named the first *A. jal[a]pana* for the Mpio. of Jalapana [sic] in Zac; the one from Queretaro I named for the city of same, thinking this perhaps clever for nomenclatural purposes. My initial mistake was clearly a typo. Would you still suggest a new name for the first *A. jal[a]pana*, or merely an erratum calling attn to same. Would appreciate your input.” He, of course, noted that a new name would be required, this provided here:

AGERATINA JALPANSERRA B.L. Turner, **nom. nov.** based upon *Ageratina jalpana* B.L. Turner, *Phytologia* 96: 13. 2014; not **A. jalpana** B.L. Turner, 1997.

The new name is derived from the Municipio Jalpan de Serra; while the name has changed, it still refers to the Mpio. of Jalpan!

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My thanks to H. Robinson and K. Gandhi for calling the error concerned to my attention, and to Guy Nesom for helpful comments, and suggesting the new name concerned.

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***Oenothera gayleana* (*Oenothera* sect. *Calylophus*, Onagraceae), a new gypsophile from Texas, New Mexico, and Oklahoma**

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ABSTRACT

A new species, *Oenothera gayleana*, is described from gypseous soils in western Texas (Panhandle and trans-Pecos areas), southeastern New Mexico, and western Oklahoma. It is closely related to *O. capillifolia* subsp. *berlandieri* of sect. *Calylophus* but is readily distinguished by its consistently longer, linear, essentially entire leaves, and restriction to bare gypseous outcrops. Photographs of plants in the field, as well as the type, are provided, along with maps showing its distribution and that of its closest congener, *O. capillifolia*. Published on-line www.phytologia.org *Phytologia* 96(3): 200-206 (July 1, 2014). ISSN 030319430

KEY WORDS: *Calylophus*, gypsum, *Oenothera*, *O. capillifolia*, *O. berlandieri*, Onagraceae, *Zeltnera maryanna*

Oenothera gayleana B. L. Turner & M. J. Moore, **sp. nov.** Figs. 1, 2.

Resembling *Oenothera capillifolia* subsp. *berlandieri* (Spach) Wagner & Hoch [= *O. berlandieri* (Spach) D. Dietr., cf. Wagner, Krakos and Hoch 2013] but the leaves linear to linear-oblongate, 10-20 times longer than wide (vs 10 times longer than wide or less), having nearly entire/slightly serrate margins (vs serrate), and the floral tubes somewhat shorter (ca. 7 mm long or less, vs longer).

Stiffly erect suffrutescent perennials 15-30(-40) cm high and about as wide, the stems decidedly ascending and arising from woody taproots with caudices 0.5-2.0 cm (occasionally to 3 cm) in diameter. **Leaves** conduplicate, linear to narrowly linear-lanceolate, not much reduced upwards, those at mid-stem mostly 25-35 mm long, 1-2 mm wide, 10-15(-20) times as long as wide, sparsely strigose to glabrous, the margins nearly entire to slightly serrate. **Sepals** 4-6 mm long, 3-4 mm wide, weakly appendaged, if at all. **Floral tubes** yellow throughout, ca. 7 mm long, ca. 5 mm across apically. **Petal lobes** yellow, 15-20 mm long, 10-15 mm wide. **Stamens** biseriate, the episealous filaments ca. 5 mm long; epipetalous filaments ca. 2 mm long; anthers 3-4 mm long. **Styles** ca. 10 mm long; stigma discoid, yellow, ca. 2 mm across. **Capsules** linear-arcuate, moderately strigose, 18-20 mm long, ca. 2 mm wide. **Seeds** oblongate, glabrous, ca. 1.5 mm long, 1.0 mm wide. **Chromosome number**, $n = 7$, including varying numbers of pairs and rings (as cited below). **Flowering** known from early May through late September.

TYPE: USA. TEXAS: Culberson Co., 13.1 mi E along FM 652 from its intersection with US 62/180, bare gypsum hills, 25 May 2004, *B. L. Turner* 24-255. (Holotype: TEX; isotypes: BRIT, MO, SRSC).

ADDITIONAL SPECIMENS EXAMINED: NEW MEXICO. De Baca Co.: Gypsum land 24 mi S of Ft. Sumner (Route 20), 17 Aug 1967, *J. B. Secor* 61 (TEX); Along NM 20, ca. 28 mi south of junction with US 80 in Fort Sumner, 34 09 17.7 N, 104 28 51.6 W; in gypsum, 12 Aug 2008, *M. J. Moore et al.* 669 (US). **Eddy Co.:** 11.4 mi S of Whites City on US 62/180, 9 May 1967, *H. F. Towner* 21 [The collector

(on the sheet itself) notes three chromosome counts in this population: $2n = 7$ pairs; $2n = 5$ pairs + 1 ring of 4 chromosomes; and $2n = 2$ pairs + 1 ring of 4 chromosomes, 1 ring of 6 chromosomes, and 2 diminutive chromosomes.]; 15 mi S of Whites City, 2 mi E along hiway 180, “Growing in pure gyp. soil,” 22 May 1967, *B. L. Turner 5664* (TEX); ca. 1 mi from Texas/New Mexico line along US 62; gypsum soils, 18 Aug 2004, *B. L. Turner 24-411* (TEX); Along US 62/180 ca. 5.2 mi N of jct w/ FM 652, 32 02 36.9 N, 104 28 10.3 W, in gypsum, 10 Aug 2008, *M. J. Moore et al. 653* (US); At east end of Seven Rivers Hills, near gravel road that leads from US 285, N of Carlsbad, 32 33 41.7 N, 104 25 33.8 W, on gypsum, 11 Aug 2008, *M. J. Moore et al. 660* (US); Along gravel road leading east from US 62/180, in Yeso Hills, 32 02 25.1 N, 104 27 25.9 W, on gypsum, 26 May 2012, *M. J. Moore 1758* (US); Yeso Hills, ca. 0.5 mi along gravel road leading east from US 62/180, 32 02 13.9 N, 104 27 18.8 W, on gypsum, 20 Aug 2013, *M. J. Moore et al. 2286* (US).

OKLAHOMA. Harmon Co.: 13.5 mi W of Mangum, gypsum, 3 Jun 1948, *U. T. Waterfall 7766* (TEX).

TEXAS. Collingsworth Co.: N side of Elm Creek canyon on Elm Creek Ranch, ca. 7.2 airmiles SE of jct. I-10 and US Rt. 83 in Shamrock, 35 08 19.6 N 100 10 16.4 W, gypsum outcrops, 2150 ft, 18 Sep 2007, *W. R. Carr et al. 26378* (TEX). **Culberson Co.:** about half [way] between El Paso-Carlsbad road [US 62/180] and Delaware Creek, 5 Jul 1952, *L. C. Hinckley 4862* (SRSC); low gyp hills, ca. 13 mi W of [Reeves] county line, 24 Jun 1970, *A. M. Powell 1921* (SRSC); 22.4 mi W of Reeves Co. line along hiway 652, gypsum knolls, 1 Aug 2000, *B. L. Turner 20-445B* (SRSC, TEX); ca. 19.5 mi E along hiway 652 from its intersection with US 62, 18 Aug 2004, *B. L. Turner 24-411* (TEX); Just N of gravel road that goes E from FM 2185, 31 21 05.5 N, 104 27 06.6 W, on gypsum, 25 Aug 2013, *M. J. Moore et al. 2354* (US). **Dickens Co.:** S of US highway 82, 24 Jun 1944, *C. L. Lundell 12979* (TEX); Croton Breaks, ca. 15 mi ESE of Dickens along gypsum ridges, 29 Sep 2004, *Turner 24-454* (SRSC, TEX); 6.4 mi S of US Hwy 82 on road to Croton Camp of the Pitchfork Ranch (i.e., 15.2 mi E from Dickens on 82, then S 3.9 mi on Co Rd 371, then SW 2.5 mi on Co Rd 366), in the Croton Breaks, 33 32 49 N, 100 37 36 W, 10 Jul 2000, *T. L. Wendt & K. Collins 7124* (TEX); Along Dickens County Road 371 ca. 0.8 mi south of junction with US 82, 33 36 12.0 N, 100 34 10.5 W, on gypsum hillside, 22 Jul 2009, *M. J. Moore et al. 790* (US).

In Towner’s (1977) biosystematic study of *Calylophus*, material of this taxon was not cited, although in his account of the “Distribution” of *C. berlandieri* subsp. *berlandieri* he alludes to collections from Culberson County, Texas; in his well-crafted treatment the present novelty will key to or near *C. berlandieri* subsp. *berlandieri* (= *O. capillifolia* subsp. *berlandieri*) and/or subsp. *pinifolia* (Engelm. ex A. Gray) Towner (= *O. capillifolia* subsp. *capillifolia*). *Oenothera gayleana* is readily distinguished from both subspecies in having longer, linear, nearly entire leaves and by its somewhat smaller flowers, as given in the above diagnosis. The reduced leaf morphology of *O. gayleana* is typical of the overwhelming majority of gypsum endemic species worldwide (Escudero et al., 2014) and may represent an edaphic adaptation to growing on gypsum; it also shares with most gypsum endemic taxa a more shrubby habit compared to its near relatives. Indeed, the taxon has a very compact, highly branched habit with a single strong caudex that can grow as much as 3 cm in diameter in older individuals.

Towner (1977) perceptively noted that “extremely narrow-leaved plants formerly known as *Oenothera serrulata* subsp. *pinifolia* are clearly variants which can actually be found along with broader-leaved plants in populations of either subspecies of *C. berlandieri*.” He further commented that his treatment was similar “to that of Shinnars (1946), who did not recognize subsp. *pinifolia*, viewing it as merely the extreme in a wide range of variation, the latter due to spontaneous mutation. The narrow-leaved plants are most frequently found in areas of highly calcareous soil, including gypsum, and they occur in the more arid portions of the range of *C. berlandieri*. Thus their presence may well be due not to spontaneous mutations, but to edaphic selection factors.” The above remarks are perceptive and presage recognition of the present taxon.

We also recognize that the present novelty might with equal validity be recognized as but a localized variety or subspecies of *O. capillifolia* subsp. *berlandieri*; indeed, Towner himself annotated the Dickens Co. populations as such (cf. *Lundell 12979*, TEX). Nevertheless, the distinctive leaves, habit, and habitat of the gypsum populations, which are maintained across a broad area of discontinuous gypsum exposures in three states (Fig. 3), are suggestive of specific status. Populations of *O. capillifolia* subsp. *berlandieri* away from gypsum are generally taller (as much as 1 m tall) with longer internodes, wider leaves, and a laxer overall habit, as for example seen in populations on the quartz sand dunes near Monahans, TX [e.g., *M. J. Moore et al. 757*, Ward County, TX (US)]. Workers in the future using DNA data might help resolve such alternatives. So far as known, it does not co-occur with subsp. *berlandieri*, nor does it appear to intergrade into contiguous populations of that taxon in regions to the north or east. The distributions of *Oenothera capillifolia* and its infraspecific taxa are shown in Fig. 4.

Throughout most of its range, *Oenothera gayleana* grows intermixed with the much larger-flowered *O. hartwegii* subsp. *filifolia*, without evidence of hybridization (cf. *Turner 20-445B*, TEX). In the northern part of its range, *Oenothera gayleana* occasionally occurs near the superficially similar *O. serrulata* Nutt. of sect. *Calylophus*, a permanent translocation heterozygote taxon (Towner, 1977) that is distinguished by its more prominently serrate leaves and generally smaller flowers.

The senior author has named the taxon for his wife of ca. 25 years, Gayle Turner (nee Langford), but now divorced, mainly because he had previously described *Centaureum maryannum* [= *Zeltnera maryanna* (B.L. Turner) Mansion; Turner, 1993] from these same gypseous outcrops, this in honor of Mary Ann Langford, Gayle's mother. The idea that both mother and daughter are endearingly eponymized as part of this localized edaphic setting gives him pleasure. It is his hope that future floristic workers will call attention to this romantic quirk, making the natural habitat concerned more memorable for such bestowal.

As an aside, the senior author takes a sort of ironic pleasure in calling the reader's attention to the fact that when he first met Gayle (while she was dating a young dentist-to-be, both enrolled in my Native Plants course at the University of Texas), her erstwhile paramour referred to her lovingly as "his little *Oenothera*." The present description makes prescient his presentiments, albeit in on the senior author's behalf; he naturally picked up on the eponym, often referring to her as "my little *Calylophus*," this prior to the resubmergence of the latter into *Oenothera*, sensu lato (Wagner et al., 2007).

Biogeographic observations. *Oenothera gayleana* forms a part of a distinctive florula of gypsophiles with a center of distribution in southeastern New Mexico and adjacent Culberson County, Texas (Waterfall, 1946). This florula includes several taxa that are restricted to this area: *Abronia nealleyi* Standley (cf. Turner, 2004; Ackerfield and Jennings, 2008), *Anulocaulis leiosolenus* (Torr.) Standl. var. *gypsogenus* (Waterf.) Spellb. & T.Wooten, *Astragalus gypsodes* Barneby, *Eriogonum gypsophilum* Wooton & Standl., *Linum allredii* R.C.Sivinski & M.O.Howard, *Senecio warnockii* Shinnery, and *Zeltnera maryanna* (B.L. Turner) Mansion. Interestingly, none of the above taxa, including *O. gayleana*, is known to grow west of the Guadalupe Mountains. Three of these taxa (*L. allredii*, *O. gayleana*, and *Z. maryanna*) have been described in the past 21 years (Turner, 1993; Sivinski and Howard, 2011), suggesting that further exploration and study of specimens already collected in the area, particularly on the large gypsum plains of the Castile Formation (e.g., King, 1948; Weber and Kottlowski, 1959), which extend almost 90 km north to south, may yield additional undescribed taxa. This is particularly true of the Texas portions of this deposit, which are much more extensive and less well explored botanically.

It is important to note that *Oenothera gayleana*, while a common and conspicuous element of the gypsum exposures in southeastern New Mexico and Culberson County, is not restricted to this area, unlike the taxa mentioned above. The disjunct populations of *O. gayleana* on the large gypsum deposits

of north Texas and western Oklahoma seem unusual at first, although other taxa restricted to gypsum in this area share similar disjunctions. The gypsophilic *Nama stevensii* C.L.Hitchc. var. *stevensii* shares a similar overall distribution to *O. gayleana*, although it extends further north into Oklahoma and southern Kansas (Tyrl et al., 1984). Likewise, *O. hartwegii* Benth. subsp. *filifolia* (Eastw.) W.L.Wagner & Hoch, another gypsophilic member of sect. *Calylophus* that is ubiquitous on gypsum throughout New Mexico and west Texas, also grows on gypsum north of Big Spring, TX (Towner, 1977). Finally, *Haploësthes greggii* var. *texana*, which is confined to gypsum throughout its distribution except for a few populations along and near the Rio Grande, is also disjunct between New Mexico/west Texas and the gypsum of north Texas, western Oklahoma, and southern Kansas (Turner, 1975).

ACKNOWLEDGEMENTS

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Fig. 1. Holotype of *Oenothera gayleana* (TEX).

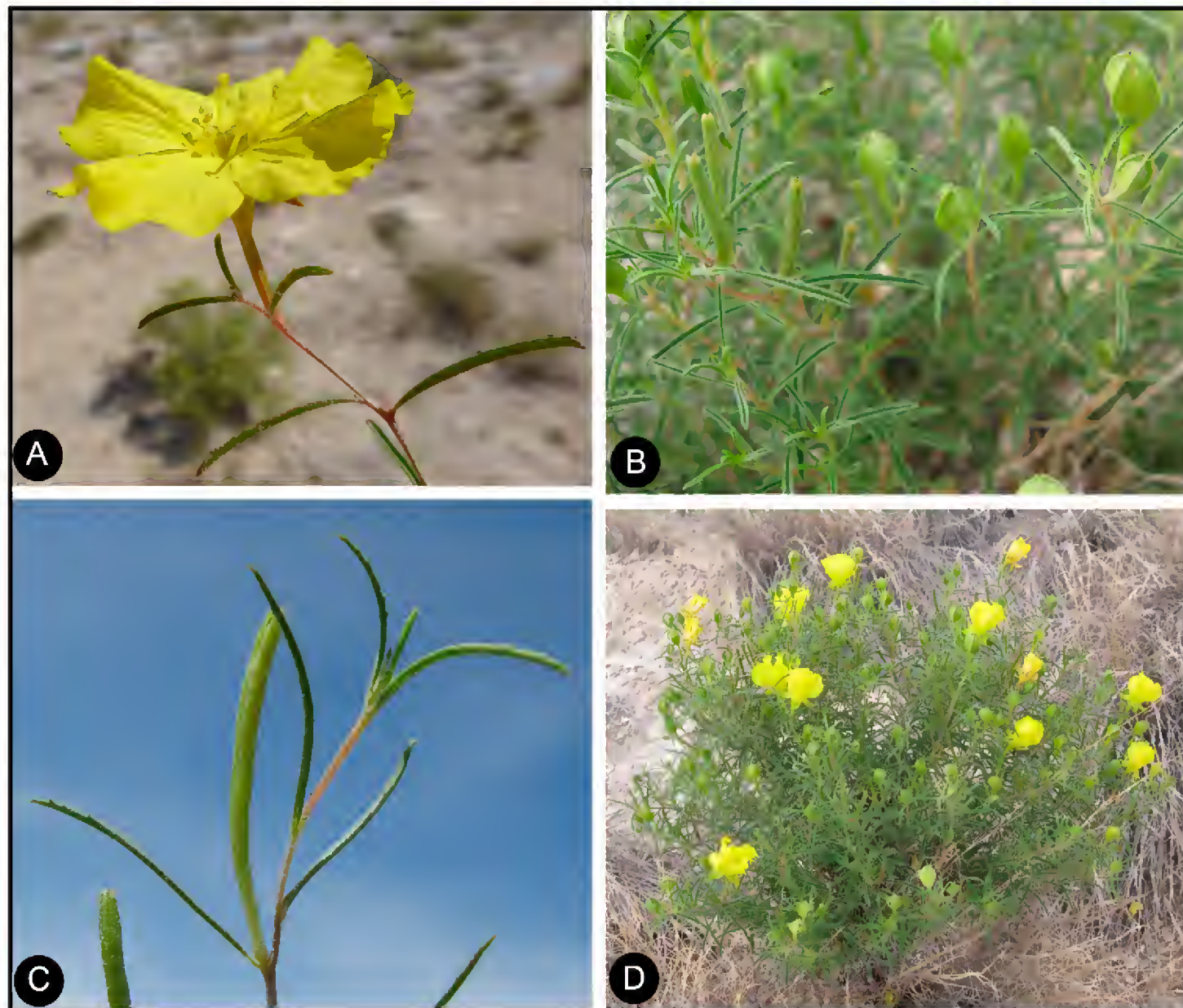


Fig. 2. Images of *Oenothera gayleana* in the field: (A) flower and leaves; (B) leaves, flower buds, and immature fruits; (C) leaves and immature fruit; (D) habit.

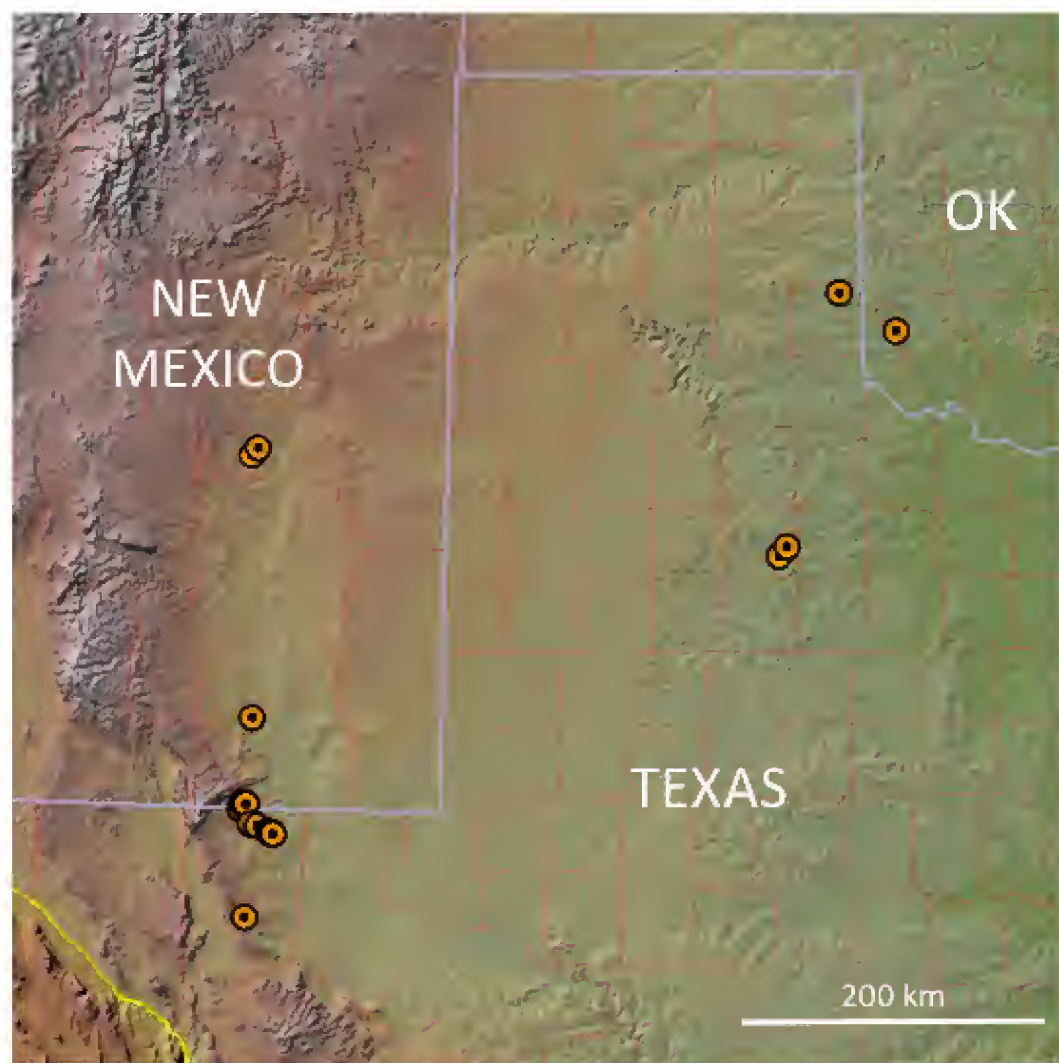


Fig. 3. Distribution of *Oenothera gayleana*.

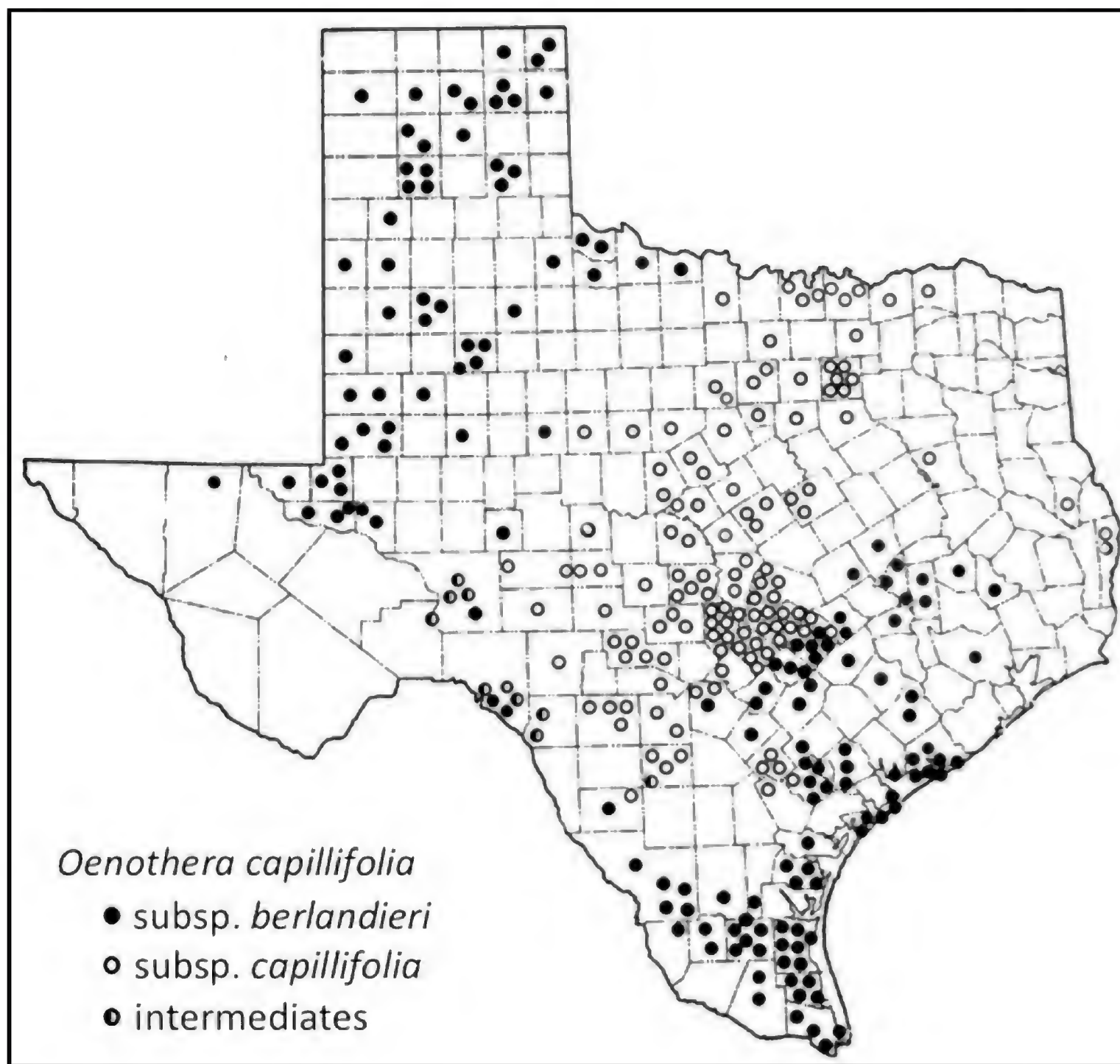


Fig. 4. Distribution of *Oenothera capillifolia* in Texas.

Comparison of volatile leaf terpenoids from *Juniperus monosperma* and *J. osteosperma* leaves: intact, ground and exposed to ambient temperature.

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ABSTRACT

The effects of sample preparation on yields and composition of volatile terpenoids were examined for oils from *Juniperus monosperma* and *J. osteosperma* leaves obtained by 24 h steam distillation from intact, ground-frozen, ground- 4h RT, and ground-18h RT leaves. For *J. monosperma*, the total oil yield was largest from ground-frozen (4.48%), then declined in the 4h RT (4.19%) and 18 h RT (2.51%) treatments, with yield from intact leaves being intermediate (3.46%). The major component, α -pinene, declined from 22.7 mg/g to 5.8 mg/g upon exposure to RT for 18h. For *J. osteosperma*, the total oil yield was also largest from the ground-frozen (8.9%), then declined in the 4h RT (5.3%) and 18 h RT (4.7%) treatments, with yield from intact leaves being intermediate (5.63%). The major component, bornyl acetate declined from 14.4 mg/g to 10.0 mg/g upon exposure to RT for 18h. Sabinene declined from 11.3 mg/ g to 3.5 mg/g after exposure to RT for 18h. The leaf oils of *J. osteosperma*, having much less volatile monoterpenes and with oil glands deeply embedded in its leaves, were much less affected by exposure to RT for 18h. Published on-line www.phytologia.org *Phytologia* 96(3): 207-217 (July 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus monosperma*, *J. osteosperma*, *Neotoma stephensi*, woodrats, terpene distillation, ground leaves, exposure to ambient.

There have been several studies on the effects of leaf storage at ambient (room temperature, RT) on the volatile leaf oil yields and composition of *Juniperus* (*J. thurifera*, Achak, et al., 2008; *J. excelsa*, Shanjani et al., 2010; *J. pinchotii*, *J. virginiana*, Adams, 2010; 2011; 2012a; 2013a, b). Adams (2012a) reported the oil yields of *J. virginiana* varied non-significantly between fresh leaves and those stored for up to 18 mo. at RT, but oil yields significantly declined between 18 and 25 mo. at RT. The major monoterpenes, sabinene and limonene, were stable for up to 8 mo., then significantly declined at 18 mo. and 25 mo. (Adams, 2012a). It might be noted that the volatile leaf oil is stored in oil glands. and the oil glands in the leaves of *J. virginiana* are embedded (sunken) in the leaves and do not rupture. In a study of *J. pinchotii*, a species with ruptured oil glands, Adams (2013b) found little significant variation in oil yields between fresh leaves and those stored up to 24 mo. at RT. The major monoterpene, sabinene, was stable for 4 mo. at RT (113 - 103 mg/g), then declined between 4 and 8 mo, then remained stable (82.2, 73.5, 80.4 mg/g) in the 8, 16 and 24 mo. at RT samples. The major oil component, camphor (ranging from 40 - 31%), declined initially from 300 mg/g (fresh leaves) to 209 mg/g (0.5 mo. at RT), then remained steady (no significant differences) from 0.5 to 24 mo. at RT (Adams, 2013b). However, all the afore-mentioned studies examined the effects of storage on volatile leaf oils of *Juniperus* using intact

leaves. None of these studies examined the effects of leaf grinding on volatile leaf oil stability during storage.

Juniperus is considered a poor forage for most mammals due to the presence of terpenes that can act as feeding deterrents (Gershenzon and Dudareva, 2007). Terpenes also have numerous toxic effects on mammals such as central nervous system depression, contact dermatitis, lung function impairment, liver and kidney cysts and even death (Sperling et al., 1967; Savolainen, 1978; Falk et al., 1990). Despite this, there are multiple species of woodrats (genus *Neotoma*) that consume juniper. *Neotoma stephensi* specializes on *Juniperus monosperma*; *N. albigula* consumes *J. monosperma* and *J. osteosperma*; and *N. lepida* consumes *J. osteosperma* (Vaughan, 1982; Torregrossa and Dearing, 2009; Magnanou et al., 2009). For example, browsing patterns on *J. monosperma* by the specialist *N. stephensi*, do not seem to be driven by terpene content (Adams et al., 2014) probably due to the animals' efficient physiological mechanisms to deal with the terpenes present (Boyle and Dearing 2003; Sorenson et al., 2004; Skopec et al., 2007; Skopec and Dearing, 2011; Torregrossa et al., 2011). Understanding the physiological and behavioral adaptations that allow these woodrat species to consume juniper may provide insight on ways to improve other mammalian species' performance on juniper. Juniper encroachment into rangelands is a major concern in the American West and increasing the voluntary intake of juniper by sheep or goats is proposed as a viable biocontrol tool (Estell et al. 2014a,b, Utsumi et al., 2013).

When feeding juniper leaves mixed with other feedstocks, under lab conditions, it is important that juniper and other feedstocks be finely ground and mixed, so woodrats (or other animals under consideration) do not intentionally select for certain feed components. However, merely grinding juniper leaves appears to release terpene volatiles as some oil glands are ruptured during grinding. In addition, as the feed is served at room temperature (RT), additional volatiles are likely lost during the course of a feeding trial. The purpose of the present paper is to report on the effects of sample preparation on yields and composition of volatile terpenoids from *Juniperus monosperma* and *J. osteosperma* leaves obtained by 24h steam distillation of leaves from four treatments: intact, ground-frozen, ground- 4h RT, and ground-18h RT.

MATERIALS AND METHODS

Plant material: Juniperus monosperma - a bulk collection was made by K. Kohl (2014) 35° 26.708' N; 111° 21.572' W, elev. 5290 ft, November, 2013, Coconino Co., AZ.

Leaf material subsamples (approx. 50 g FW) treated as: (Adams lab accession)

Adams 14209, intact fresh leaves.

Adams 14210, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, then frozen immediately.

Adams 14211, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 4h, then frozen.

Adams 14212, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 18h, then frozen.

J. osteosperma - a bulk collection was made by K. Kohl (2014) 40° 19'N 112° 54'W, 5650 ft, White Rocks, Tooele Co., UT.

Leaf material subsamples (approx. 50 g FW) treated as: (Adams lab accession)

Adams 14213, intact fresh leaves.

Adams 14214, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, then frozen immediately.

Adams 14215, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 4h, then frozen.

Adams 14216, fresh leaves, ground with dry ice in a 4L stainless steel laboratory grade Waring blender to pass 1mm sieve, exposed to RT, 18h, then frozen.

Essential oils analysis - A portion (50 g FW) of the fresh foliage was kept cold (-20°C) and in the dark; then the leaves with 2 mg of methyl decanoate added (as an internal standard) were exhaustively steam-distilled for 24 h using a modified circulatory Clevenger-type apparatus (Adams 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C until analyzed. Steam distilled leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt. / (oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 μ l of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 220°C, detector 240°C, scan time 1/sec, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

Statistical analyses - Terpenoids (as percentage of total oil and as mg per g dry foliage weight) were compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Differences were considered significant at $P \leq 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

Comparisons between the yields and compositions of intact leaves, ground-frozen, ground-4h RT, and ground-18 RT materials are given in Table 1. Notice the oils are much easier to distill from ground-frozen than intact leaves (3.26% intact, 4.48%, ground-frozen). In *Juniperus*, the leaf oil is sequestered in oil glands (Fig. 1). The oil glands of *J. monosperma* are near the leaf surface as in *J. californica* and *J. occidentalis* (Fig. 1). It is not surprising that ground leaves release the oils more readily than intact leaves.

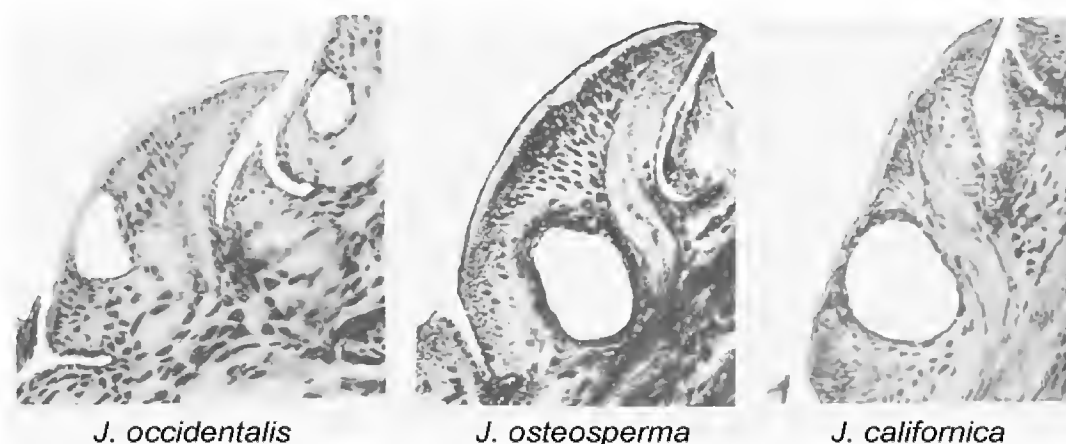


Figure 1. Leaf cross-sections for *J. occidentalis*, *J. osteosperma* and *J. californica* (from Frank Vasek, pers. communication). Notice for *J. occidentalis*, which has conspicuous and ruptured oil glands, the gland is at the leaf surface. The oil glands of *J. californica* are conspicuous, and occasionally ruptured. The oil glands in *J. osteosperma* are not conspicuous nor ruptured and are embedded in the leaves. The oil glands of *J. monosperma* (not shown) are near the leaf surface as in *J. californica*, conspicuous and often ruptured.

Oil yields and α -pinene (% data) show (Fig. 2) similar patterns. Oil and α -pinene yields are both larger in ground leaves than intact leaves. Comparing ground-frozen, ground-4h RT and ground-18h RT shows a significantly different decline between ground-4h RT and ground-18h RT treatments. This same pattern was observed in the other monoterpenes. Examination of yields and α -pinene (mg/g basis) shows a similar pattern (Fig. 3) to the % data (Fig. 2), except the decrease in α -pinene in the mg/g data is not nearly as severe as in the % data.

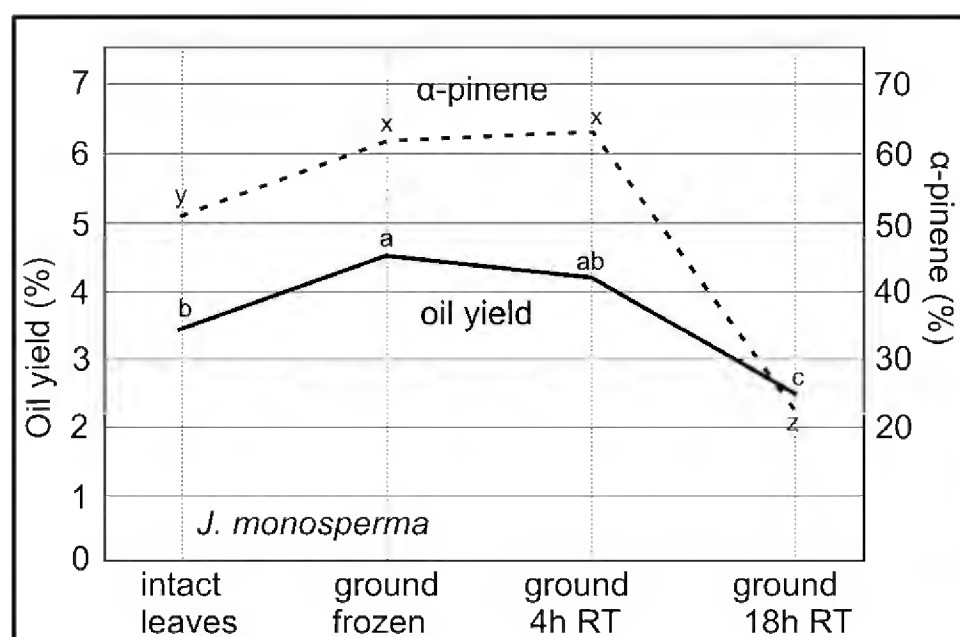


Fig. 2. Oil yields and α -pinene (% total oil, DW basis) of *J. monosperma*. Any data points on a line with a different letter are significantly different.

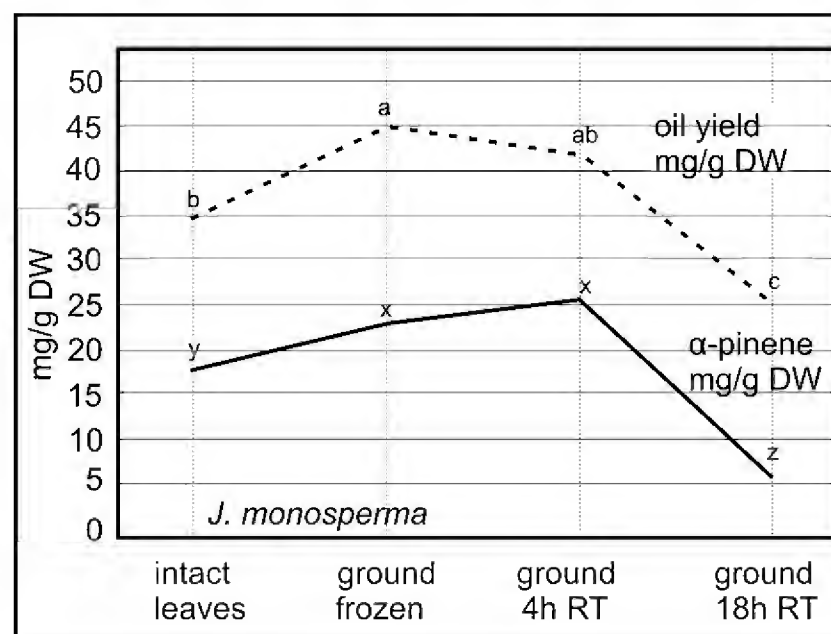


Fig. 3. oil and α -pinene of *J. monosperma*. (mg/g DW basis)

The β -eudesmol and elemol, sesquiterpenes, being less volatile than monoterpenes, displayed the opposite pattern (Fig. 4, % data). The decline in these sesquiterpenes from intact leaves and ground-frozen leaves appears to be due to the higher efficiency of removal of the more volatile monoterpenes from the ground-frozen leaves. The concentration of the sesquiterpenes was relatively larger in the ground-18h RT sample due to the loss of the more volatile monoterpenes during exposure of 18h at RT. A similar pattern is seen on a mg/g DW basis (Fig. 5), but less of a change between 4h RT, and 18h RT as β -eudesmol and elemol are not very volatile.

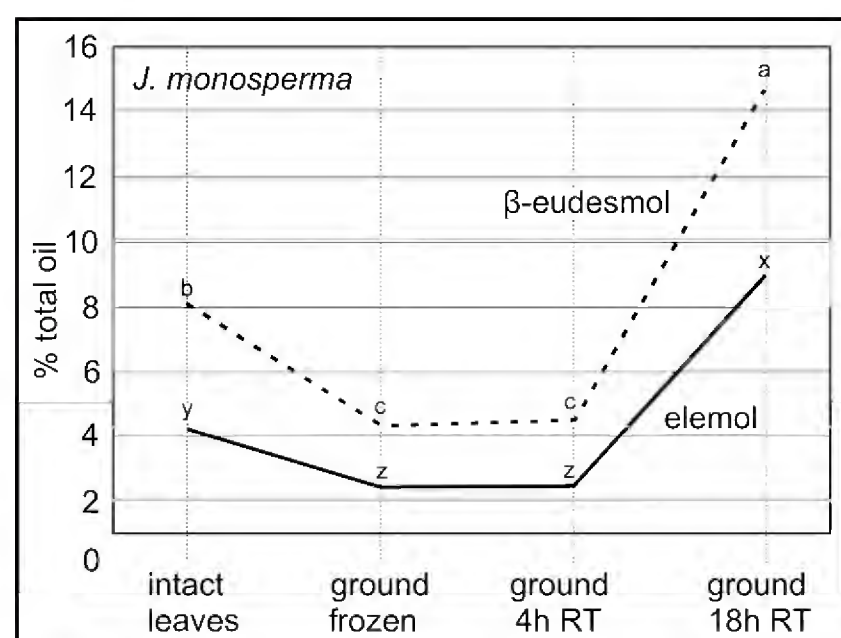


Fig. 4. Changes in β -eudesmol and elemol in the treatments. Note the large increase in the ground-18h RT sample. (% total oil basis)

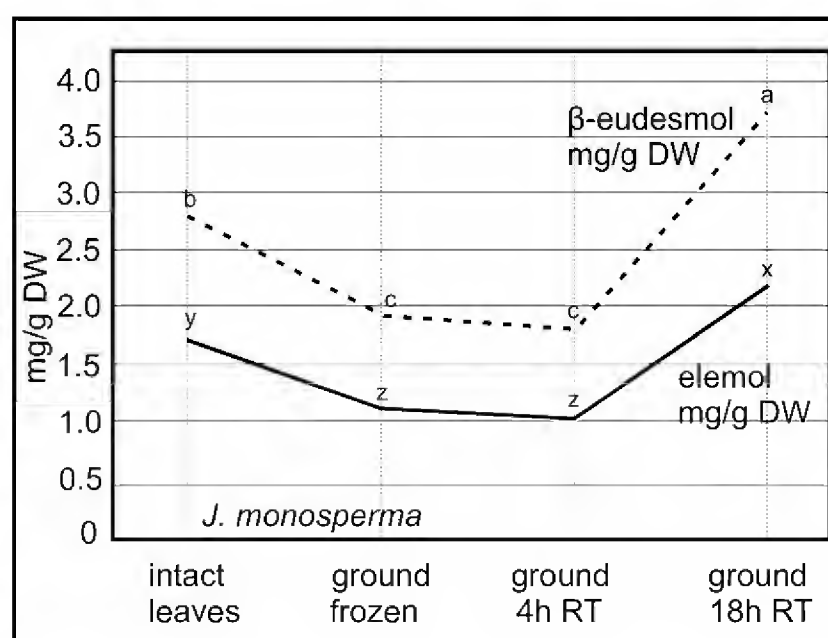


Fig. 5. Graph of β -eudesmol and elemol (mg/g DW basis). The increase from ground-4h RT to 18h RT is less than in the % total oil data (Fig. 4).

Although leaf browning (or yellowing) is visible after 4h RT (Fig. 6), the browning is more advanced in the 18h RT sample (Fig. 6). The browning is likely due to the oxidation of phenolics by polyphenol oxidase. However, no new components were found in the leaf oils (Table 1). It appears that the terpenoids extracted in this study are not susceptible to this type of oxidation and exposure to RT for up to 18h does not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.



Fig. 6. Colors of ground-frozen, ground-4h RT and ground-18h RT leaves.

The composition of the oil from intact leaves of *J. osteosperma*, Utah, is shown in Table 2. It might be noted that the composition of this oil differed somewhat from the recent leaf oil report (Adams, 2012b, 2h distillation). This is likely due to difference in distillation time (24h vs. 2h), and geographic variation in *J. osteosperma* leaf oils. Yet, it is notable that the present *J. osteosperma* oil contained only a small amount of camphor (5.1%) compared to 16 to 60% camphor reported by Adams (2012b).

The trend in oil yields for *J. osteosperma* (Table 2) is similar to that found for *J. monosperma* (Table 1) with intact leaves yielding less oil than ground-frozen leaves (5.63 vs. 8.90%, Table 2). However, there is very large drop in oil yields from ground-frozen (8.90%) to ground-4h RT (5.30%) and ground-18h RT (4.7%), compared to a modest decline in *J. monosperma* from the ground-frozen (4.48%) to the ground-4h RT (4.19%). Because the oil glands of *J. osteosperma* are embedded in the leaves (Fig. 1), this could account for the higher efficiency of oil distillation from ground leaves (8.90%) than from intact leaves (5.63%).

Yields for α -pinene and sabinene show the same pattern for the four treatments: an increase in the ground-frozen leaves, then a decline with exposure to RT conditions (Fig. 7, % total oil basis). A similar pattern is seen on a mg/g DW basis (Fig. 8), except α -pinene, and sabinene show a greater decline from ground-frozen to ground-4h RT treatments than found in the % total oil data (Fig. 7).

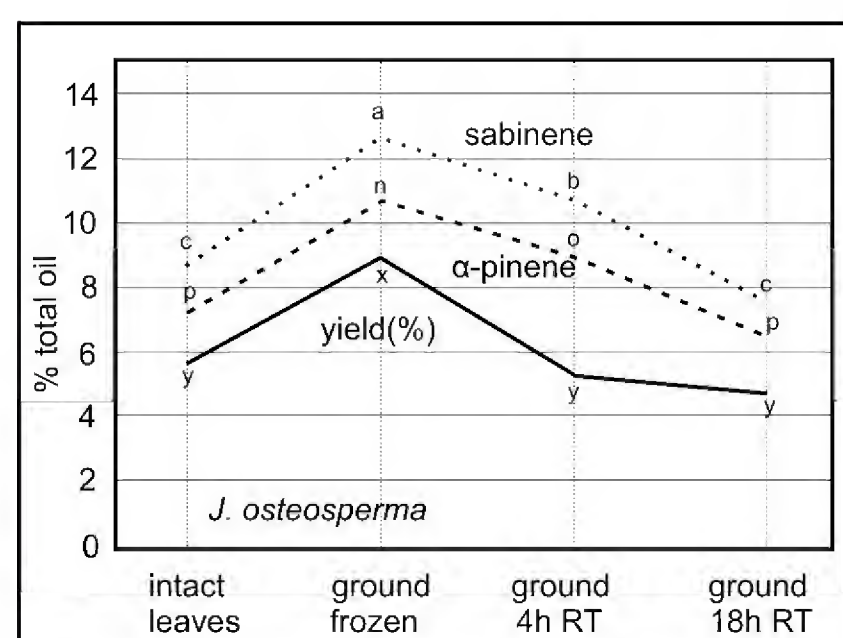


Fig. 7. Changes in oil yield, α -pinene, and sabinene (% total oil basis).

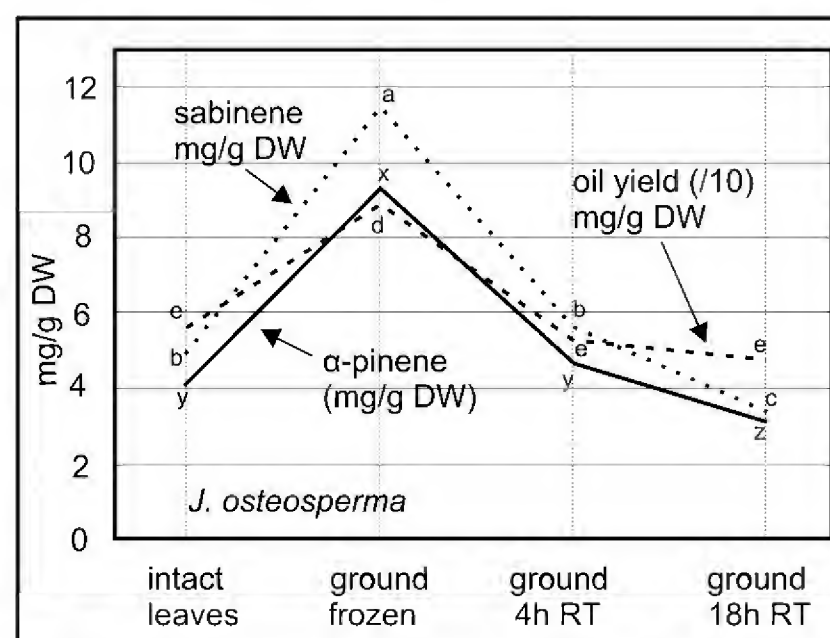


Fig. 8. Variation in oil yield, α -pinene, and sabinene (mg/g DW basis).

The major oxygenated terpenoids and sesquiterpenoids, bornyl acetate, camphor and elemol, display a different pattern (Fig. 9, % total oil basis). Intact leaves yielded more of these compounds,

decreasing in the ground-frozen leaf extract (Fig. 9). Bornyl acetate and camphor both increased as a proportion the total oil upon exposure to RT (Fig. 9). However, on a mg/g DW basis, larger amounts bornyl acetate and camphor were obtained from ground-frozen samples than from intact leaves. This, of course, reflects the overall greater yields obtained from ground leaves (Fig. 7). It is interesting that the yields of elemol, a less volatile sesquiterpene alcohol, was not much influenced by grinding or exposure to RT conditions (Figs. 9, 10).

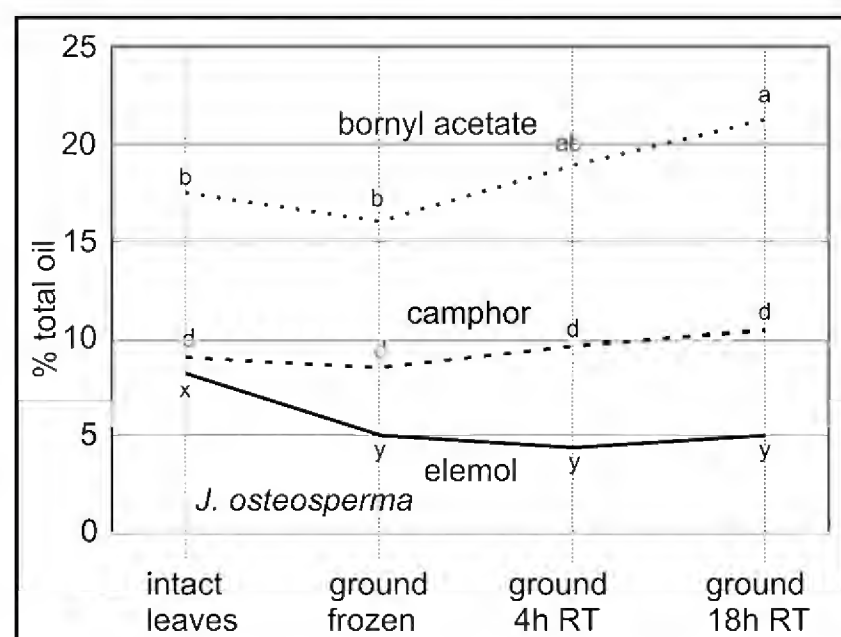


Fig. 9. Variation in bornyl acetate, camphor and elemol (% total oil basis) among treatments.

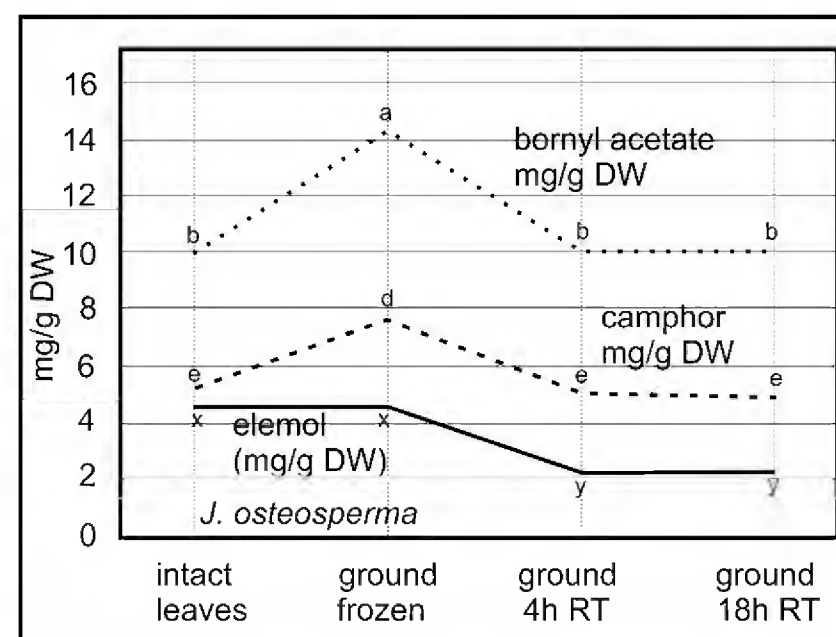
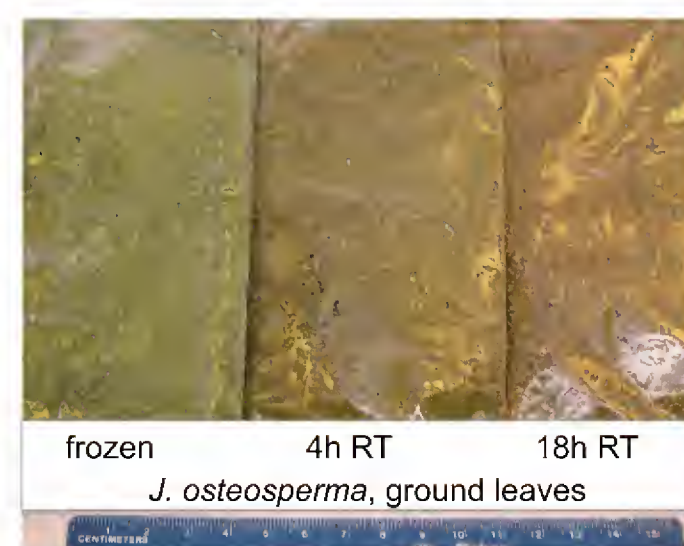


Fig. 10. Changes in bornyl acetate, camphor and elemol (mg/g DW basis).

The volatile leaf terpenoids of *J. osteosperma*, as previously seen in *J. monosperma*, are impacted by exposure to RT conditions for 4h and 18h. However, on a mg/g DW basis, the amount of terpenes lost from *J. osteosperma* ground leaves appears to be less than found in *J. monosperma*. This seems likely due to the embedded oil glands and the lesser amounts of volatile monoterpenes in *J. osteosperma* (43.64%) vs. *J. monosperma* (78.50%). Thus, the *J. osteosperma* oil is much less volatile than that of *J. monosperma* and coupled with the leaf glands being deeply embedded in the leaves (Fig. 1), leads to less loss of the individual components when exposed to RT conditions than found in *J. monosperma*. It is likely that if woodrats cache *J. osteosperma* leaves in their middens, those leaves will not lose their oils as quickly as leaves of *J. monosperma*.

Examination of the colors of the leaves of *J. osteosperma* shows yellowing of the 4h RT and 18h RT treatments (Fig. 11) that are very similar to that seen in the *J. monosperma* leaves (Fig. 6). As in the case of *J. monosperma* leaves, exposure to RT for up to 18h does not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.

Fig. 11. Colors of ground-frozen, ground-4h RT and ground-18h RT *J. osteosperma* leaves.



CONCLUSION

The effects of sample preparation on yields and composition of volatile terpenoids were examined for oils from *Juniperus monosperma* and *J. osteosperma* leaves, obtained by 24 h steam distillation from intact, ground-frozen, ground- 4h RT, and ground-18h RT leaves. For *J. monosperma*, the total oil yield was largest from the ground-frozen (4.48%), then declined in the 4h RT (4.19%) and

18h RT (2.51%), with yield from intact leaves being intermediate (3.46%). The major component, α -pinene, declined from 22.7 mg/g to 5.8 mg/g upon exposure to RT for 18h. For *J. osteosperma*, the total oil yield was also largest from the ground-frozen (8.9%), then declined in the 4h RT (5.3%) and 18 h RT (4.7%) treatments, with yield from intact leaves being intermediate (5.63%). The major component, bornyl acetate, declined from 14.4 mg/g to 10.0 mg/g upon exposure to RT for 18h. Sabinene declined from 11.3 mg/g to 3.5 mg/g after exposure to RT for 18h.

The loss of volatile terpenes appears to be mostly effected by differences in total amounts of volatile monoterpenes in *J. osteosperma* (43.64%) and *J. monosperma* (78.50%) and the position of the oil glands (*J. osteosperma*, deeply embedded in the leaves vs. *J. monosperma*, near the leaf surface). *Juniperus monosperma*, with more volatile leaf oil and oil glands near the leaf surface, was much more affected by exposure to RT for 18h than *J. osteosperma* with deeply embedded oil glands, and less volatile oil.

Interestingly, even though the ground leaves were yellowed (brownish) by exposure to RT for up to 18h, this did not seem to induce any artifacts into the oil, except for the loss of the more volatile monoterpenes.

The differences in terpene content and volatility between *J. monosperma* and *J. osteosperma* may help explain differences seen in the woodrat species' tolerance to and preference for specific juniper species.

ACKNOWLEDGEMENTS

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Table 1. Comparison of *J. monosperma* leaf oils obtained from leaves that were: intact, ground-frozen, ground-4h RT and ground-18h RT. signif. = significance level, P 0.05 = *, P 0.01 = **; ns = non significant, nt = not tested. Data values on a line that share a common letter are not significantly different. Components in boldface are data on a mg/g DW basis.

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	signif.
	oil yields, 24h dist. - % DW	3.46% b	4.48% a	4.19% ab	2.51% c	**
	oil yields, 24h dist. - mg/g DW	34.6 b	44.8 a	41.9 ab	25.1 c	**
921	tricyclene values as % total oil	0.1%	0.2%	0.2%	0.1%	nt
924	α -thujene	t	t	t	t	nt
932	α -pinene	50.8 b	61.8 a	63.3 a	22.9 c	**
	α-pinene, mg/g DW	17.5 b	22.7 a	25.5 a	5.8 c	**
945	α -fenchene	0.1	0.1	0.1	t	nt
946	camphene	0.3	0.3	0.3	0.1	nt
969	sabinene	0.1	0.1	0.1	0.1	nt
974	β -pinene	0.9 a	0.9 a	0.9 a	0.4 b	**
988	myrcene	1.4 a	1.6 a	1.5 a	0.9 b	**
1001	δ -2-carene	0.1	0.1	0.1	0.1	nt
1002	α -phellandrene	0.7 a	0.7 a	0.7 a	0.5 b	*
1008	δ -3-carene	2.1 a	2.4 a	2.5 a	1.3 b	**
1014	α -terpinene	0.1	0.1	0.1	0.1	nt
1020	p-cymene	0.3	0.3	0.3	0.2	nt
1024	limonene	2.0 a	2.1 a	1.9 a	1.2 b	**
1025	β -phellandrene	6.0 a	5.9 a	5.6 a	3.7 b	**
1044	(E)- β -ocimene	0.3	0.3	0.3	0.2	nt
1054	γ -terpinene	0.5	0.5	0.5	0.4	nt
1086	terpinolene	1.1 a	1.1 a	1.0 a	0.8 b	*
1100	linalool	t	t	t	0.1	nt
1122	cis-p-menth-2-en-1-ol	0.2	0.1	0.1	0.3	nt
1136	trans-p-menth-2-en-1-ol	0.2	0.1	0.1	0.2	nt
1141	camphor	0.3	0.3	0.2	0.4	nt
1165	borneol	t	t	t	0.2	nt
1174	terpinen-4-ol	0.3	0.3	0.2	0.5	nt
1186	α -terpineol	0.5 b	0.3 c	0.3 c	0.7 a	**
1207	trans-piperitol	0.1	0.1	t	0.2	nt
1249	piperitone	0.1	t	t	0.2	nt
1274	pregeijerene B	2.2 a	1.7 b	1.3 c	1.8 b	**
1284	bornyl acetate	0.4 b	0.5 b	0.4 b	0.8 a	**
1289	thymol	t	t	t	0.2	nt
1396	duvalene acetate	t	t	t	0.1	nt
1417	(E)-caryophyllene	0.2	0.4	0.4	0.7	nt
1452	α -humulene	0.1	0.2	0.2	0.4	nt
1489	β -selinene	t	t	t	0.2	nt
1498	α -selinene	t	t	t	0.1	nt
1500	α -muurolene	t	t	t	0.3	nt
1517	nootkatene	0.4	0.3	0.3	1.0	nt
1533	trans-cadina-1,4-diene	0.2	0.2	0.2	0.7	nt
1548	elemol	4.1 b	2.4 c	2.4 c	8.9 a	**
	elemol mg/g DW	1.7 b	1.1 c	1.0 c	2.2 a	**
1566	germacrene B	0.4 b	0.4 b	0.4 b	1.2 a	**
1629	eremoligenol	t	t	t	0.2	nt
1630	γ -eudesmol	3.1 b	2.4 bc	2.2 c	7.3 a	**
1640	epi- α -muurolol	0.2	0.2	0.1	0.6	nt
1649	β -eudesmol	8.1 b	4.3 c	4.4 c	14.6 a	**
	β-eudesmol mg/g DW	2.8 b	1.9 c	1.8 c	3.7 a	**
1652	α -eudesmol	4.7 b	3.3 c	3.2 c	11.7 a	**
1668	1-propanone, 1-(2,4-dimethoxy phenyl-)	0.3 b	0.2 c	0.2 c	0.7 a	**
1792	8- α -acetoxylemol	4.4 b	2.5 c	2.8 c	6.8 a	**

Table 2. Comparison of *J. osteosperma* leaf oils obtained from leaves that were: intact, ground-frozen, ground-4h RT and ground-18h RT. F signif. = F significance, P= 0.05 = *; ns = non significant, nt = not tested. Components in boldface are data on a mg/g DW basis.

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	F signif.
	oil yields, 24h dist. - % DW	5.63% b	8.90% a	5.30% b	4.70% b	**
	oil yields, 24h dist. - mg/g DW	56.3 mg b	89.0 mg a	53.0 mg b	47.0 mg b	**
921	tricyclene	0.9 b	1.34 a	1.1 b	0.9 b	**
924	α -thujene	0.6	0.8	0.7	0.5	nt
932	α -pinene	7.3 c	10.5 a	8.9 b	6.6 c	**
	α-pinene, mg/g DW	4.1 b	9.3 a	4.7 b	3.1 c	**
946	camphene	1.0 ab	1.3 a	1.2 ab	0.9 b	*
953	thuja-1,4-diene	0.1	0.1	0.1	0.1	nt
969	sabinene	8.6 c	12.7 a	10.7 b	7.4 c	**
	sabinene, mg/g DW	4.8 b	11.3 a	5.7 b	3.5 c	**
974	β -pinene	0.2	0.2	0.2	0.2	nt
988	myrcene	2.5	2.9	3.0	2.6	ns
1002	α -phellandrene	0.2	0.3	0.3	0.3	nt
1008	δ -3-carene	0.1	0.2	0.2	0.2	nt
1014	α -terpinene	1.8 a	1.2 b	1.6 a	1.5 a	**
1020	p-cymene	1.5	1.3	1.5	1.5	ns
1024	limonene	3.9	4.1	4.4	4.1	ns
1025	β -phellandrene	2.5	2.8	3.0	2.7	ns
1044	(E)- β -ocimene	0.2	0.3	0.3	0.3	nt
1054	γ -terpinene	3.3 a	2.1 b	2.8 a	2.8 a	**
1065	cis-sabinene hydrate	0.7	0.4	0.2	0.3	nt
1086	terpinolene	1.4	1.1	1.3	1.3	ns
1098	trans-sabinene hydrate	0.9	0.5	0.4	0.5	nt
1102	isopentyl-isovalerate	0.3	0.2	0.2	0.2	nt
1112	methyl butanoate, 3-methyl-3-butenyl-, 3-	0.4	0.3	0.3	0.3	nt
1122	cis-p-menth-2-en-1-ol	0.6	0.4	0.4	0.4	nt
1141	camphor	9.0	8.5	9.6	10.5	ns
	camphor	5.1 b	7.6 a	5.1 b	4.9 b	**
1145	camphene hydrate	0.6	0.5	0.6	0.7	nt
1154	sabina ketone	0.3	0.3	0.3	0.4	nt
1165	borneol	1.6 b	1.7 b	1.8 b	2.5 a	**
1174	terpinen-4-ol	8.6 a	5.1 c	6.6 b	7.4 b	**
1179	p-cymen-8-ol	0.4	0.3	0.3	0.3	nt
1186	α -terpineol	0.5	0.3	0.4	0.4	nt
1204	verbenone	0.6	0.4	0.5	0.5	nt
1215	trans-carveol	0.5	0.4	0.4	0.6	nt
1239	carvone	0.5	0.2	0.2	0.3	nt
1284	bornyl acetate	17.5 b	16.1 b	18.8 ab	21.3 a	*
	bornyl acetate, mg/g DW	9.9 b	14.3 a	10.1 b	10.0 b	**
1325	p-mentha-1,4-dien-7-ol	0.5	0.3	0.4	0.5	nt
1417	(E)-caryophyllene	t	0.3	0.3	0.3	nt
1451	trans-muurolo-3,5-diene	t	0.1	0.1	0.1	nt
1452	α -humulene	t	0.1	0.1	0.1	nt
1480	germacrene D	t	0.1	0.1	0.1	nt
1500	α -muurolene	t	0.1	0.1	0.1	nt
1513	γ -cadinene	t	0.1	0.1	0.1	nt
1522	δ -cadinene	0.6	0.9	0.8	0.9	nt
1548	elemol	8.2 a	5.2 b	4.2 b	4.8 b	**
	elemol, mg/g DW	4.6 a	4.6 a	2.2 b	2.3 b	**
1559	germacrene B	t	0.1	0.1	0.1	nt
1574	germacrene D-4-ol	t	0.3	t	0.1	nt
1630	γ -eudesmol	0.6 b	0.9 a	1.0 a	1.0 a	**
1647	cubenol	0.6	0.6	0.5	0.6	nt

KI	component tested	intact leaves	ground, frozen, %	ground, 4h, RT	ground 18 h RT	F signif.
1649	β-eudesmol	0.6	0.7	0.6	0.7	nt
1652	α-eudesmol	0.7	0.7	0.6	0.7	nt
1652	α-cadinol	0.6	0.7	0.6	0.7	nt
2087	abietadiene	t	0.4	0.3	0.3	nt
2315	abieta-7,13-dien-3-one	4.7 a	4.9 a	3.8 b	4.8 a	*

Confirmation of the southern-most population of *Juniperus seravschanica* in Oman by DNA sequencing of nrDNA and four cpDNA regions.

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ABSTRACT

DNA sequencing of nrDNA, plus four cp DNA regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF of putative *J. excelsa* subsp. *polycarpus* from Oman, identified the taxon as *J. seravschanica* which is closely allied with northern populations of *J. seravschanica* in Iran, Pakistan and Kazakhstan. The Oman population, the southern-most population of *J. seravschanica*, may be a Pleistocene relict or the result of more recent long-distance dispersal. Additional populational study will be needed to distinguish between these scenarios. Published on-line www.phytologia.org *Phytologia* 96(2): 218-224 (July 1, 2014). ISSN 030319430

KEY WORDS: *Juniperus seravschanica*, *J. excelsa*, *J. polycarpus* var. *polycarpus*, *J. polycarpus* var. *turcomanica*, Oman, DNA sequencing, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF.

Juniperus excelsa M.-Bieb. grows from Greece to Turkey and perhaps as far east as Azerbaijan (Fig. 1). Farjon (2005, 2010) treated *J. polycarpus*, *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpus*. However, Adams and Schwarzbach (2012) and Adams (2013), utilizing DNA sequence data, recognized *J. excelsa* in addition to *J. polycarpus* var. *polycarpus*, *J. p.* var. *turcomanica* and *J. seravschanica*. Adams and Hojjati (2012) and Adams, Hojjati and Schwarzbach (2014), using sequences from 4 gene regions, failed to verify the occurrence of *J. excelsa* in Iran, but did find *J. polycarpus*, *J. p.* var. *turcomanica* and *J. seravschanica* in Iran (Fig. 1). Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had none or only one SNP difference compared with *J. polycarpus* var. *polycarpus* from Armenia and was concluded to be *J. polycarpus* (Adams and Hojjati, 2012).

It is difficult to distinguish *J. excelsa*, *J. polycarpus* and *J. seravschanica*. The distribution of *J. excelsa* into Armenia, Azerbaijan and Iran has proved difficult to determine by modern methods of DNA sequencing, due to the lack of access to these regions. Recently, materials were obtained of *J. excelsa*/ *J. polycarpus* from Lebanon and *J. excelsa*/ *J. polycarpus* from Azerbaijan. Adams et al. (2014) found that putative *J. excelsa* from Azerbaijan was *J. polycarpus*, and unusual trees of putative *J. excelsa* at El Njass and Aarsal, Lebanon (Douaihy et al, 2011; 2013), was identified as *J. polycarpus* using DNA sequence data (Fig. 1).

In the Flora of the Arabian Peninsula and Socotra (Miller and Cope, 1996), *Juniperus excelsa* M.-Bieb. subsp. *polycarpus* (K. Koch) Takht. (treated as *J. polycarpus* K. Koch in Adams, 2014) is listed as growing in the western Al Hajar Mountains, southwest of Muscat at 1450-3000 m. Due to the proximity of the Hajar mountains to *J. seravschanica* in the Khabr mountains in Iran (Fig. 1), it seemed the juniper from Oman might be *J. seravschanica*. The purpose of the paper is to examine nrDNA, and 4 cp DNA

regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF to establish the identity of the Al Hajar juniper of Oman.

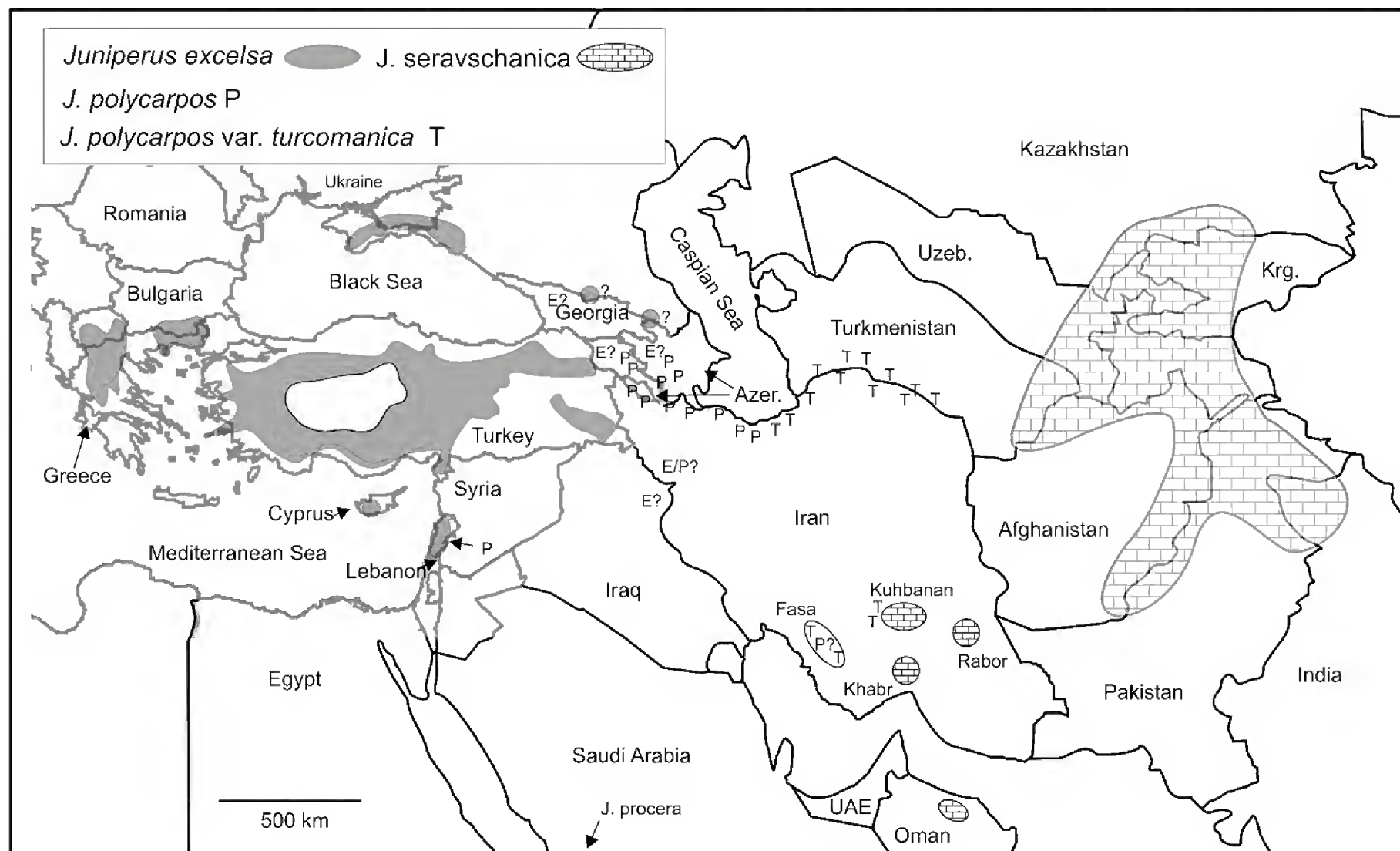


Figure 1. Distribution of *J. excelsa*, *J. polycarpos* var. *polycarpos* (P) and *J. p.* var. *turcomanica* (T) . Questionable locations of *J. excelsa* and *J. polycarpos* are indicated by E? and P? (modified from Adams, 2014).

MATERIALS AND METHODS

J. excelsa: Eskisehir, Turkey, 820m, *Adams* 9433, 9435, Lemos, Greece, 1100m, *Adams* 8785, 8786, Afqa, Lebanon, 1306 m, *Adams* 14155-14157, (=Bouchra Douaihy 1-3), 34° 04' 58.12"N, 35° 53' 08.52"E, 4 Nov 2013,

J. polycarpos: Armenia, Lake Sevan, 1900m, *Adams* 8761-8763; Azerbaijan, 177-231m, *Adams* 14161-14162 (=Vahid Farzaliyev 1-10), 40° 44' 41.05" N; 47° 35' 19.14" E, Dec 2013; Lebanon, Wadi El Njass, 2287m, *Adams* 14158-14160, (=Bouchra Douaihy 4-7), 34° 20' 47.79"N, 36° 05' 45.54"E, 14 Nov 2013; Fasa, Iran, *Adams* 13756, *Hojjati*,

J. polycarpos var. *turcomanica*: Kopet Mtns., Turkmenistan, *Adams* 8757, 8758; Fasa, Iran, *Adams* 13757, 13758 (=Hojjati ns), Oct. 2012, 29° 09' 57.8" N, 53° 40' 7.8" E,

J. procera: Guder, Ethiopia, *Adams* 6184-6188,

J. seravschanica: Oman: Amina Al-Farsi, 562-564, RPA lab acc. 14203-14205, 23° 07' 41" N, 57° 36' 9.3" E, 2340 m, northern Hajar Mtns., Jebel Al Akhdar, Da'an Al Pesaiten; Dzhabagly, Kazakhstan, *Adams* 8224-8226; Pakistan, *Adams* 8483-8485; Kuhbanan, Iran *Adams* 13760, 13761, (=Hojjati ns), Oct. 2012, 31° 28' 21.5" N, 55° 52' 58.9" E; Rabor, Iran, *Adams* 13775, 13777, (=Hojjati ns), Oct. 2012, 28° 49' 06.7" N; 56° 21' 21.7" W. elev. 2086 m; Khabr, Iran, *Adams* 13771, 13772 (=Hojjati ns), Oct. 2012, 28° 51' 8.4" N, 56° 22' 51.7" E, possible hybrid (Adams, Hojjati and Schwarzbach (2014),

J. virginiana (out-group): Knoxville, TN, USA, *Adams* 10231, 10232.

Voucher specimens deposited in the Herbarium, Baylor University (BAYLU) and Sultan Qaboos University (SQU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp regions (petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF) yielded 4430 bp of data. The Bayesian consensus tree (Fig. 2) shows *J. seravschanica*, *J. polycarpos*, *J. p. var. turcomanica*, *J. procera* and *J. excelsa* in well-supported clades. The Oman junipers are in a well-supported clade with *J. seravschanica*, nested close to Khabr and Rabor, Iran (Fig. 2). Khabr is the nearest population of *J. seravschanica* to Oman, and Rabor is the next nearest population (Fig. 1). It seems likely that during the last Pleistocene ice advance (ending ~ 15k ybp), populations of *J. seravschanica* in this region expanded their ranges into lower, cooler areas. If so, the range of *J. seravschanica* may have been nearly continuous from Pakistan to Rabor, to Khabr. Thence by long-distance dispersal (common for the establishment of *Juniperus* on islands, Adams, 2014) it became established in the Al Hajar Mtns. of Oman. Kurschner (1998) listed the Omani *Juniperus* among some other taxa of Irano-Turanian origin and described them as invaders in Arabia. Of course, the population in the Al Hajar Mtns. may be a relict from an ancient distribution. Additional studies comparing the genetic structure of *J. seravschanica* populations from Pakistan, southern Iran and Oman will be needed to determine if the Oman population is relictual or a more recent population, established by long distance dispersal.

Juniper trees in this region have the typical round to pyramidal shaped crowns (Fig. 3) of *J. seravschanica*. Even at high elevations (e.g., 2340 m at collection site), the area is semi-desert (Fig. 3) with very low precipitation. Pollen appears to be shed in Dec - Feb, mainly because one can see very small (either recently or not yet pollinated) seed cones (insert, Fig. 4). Adams (2014) notes that pollen is shed in *J. seravschanica* in fall-winter. However, those observations were based on field notes from his Kazakhstan collections. It is likely that Oman junipers shed pollen somewhat later than found in the much colder region of Kazakhstan.

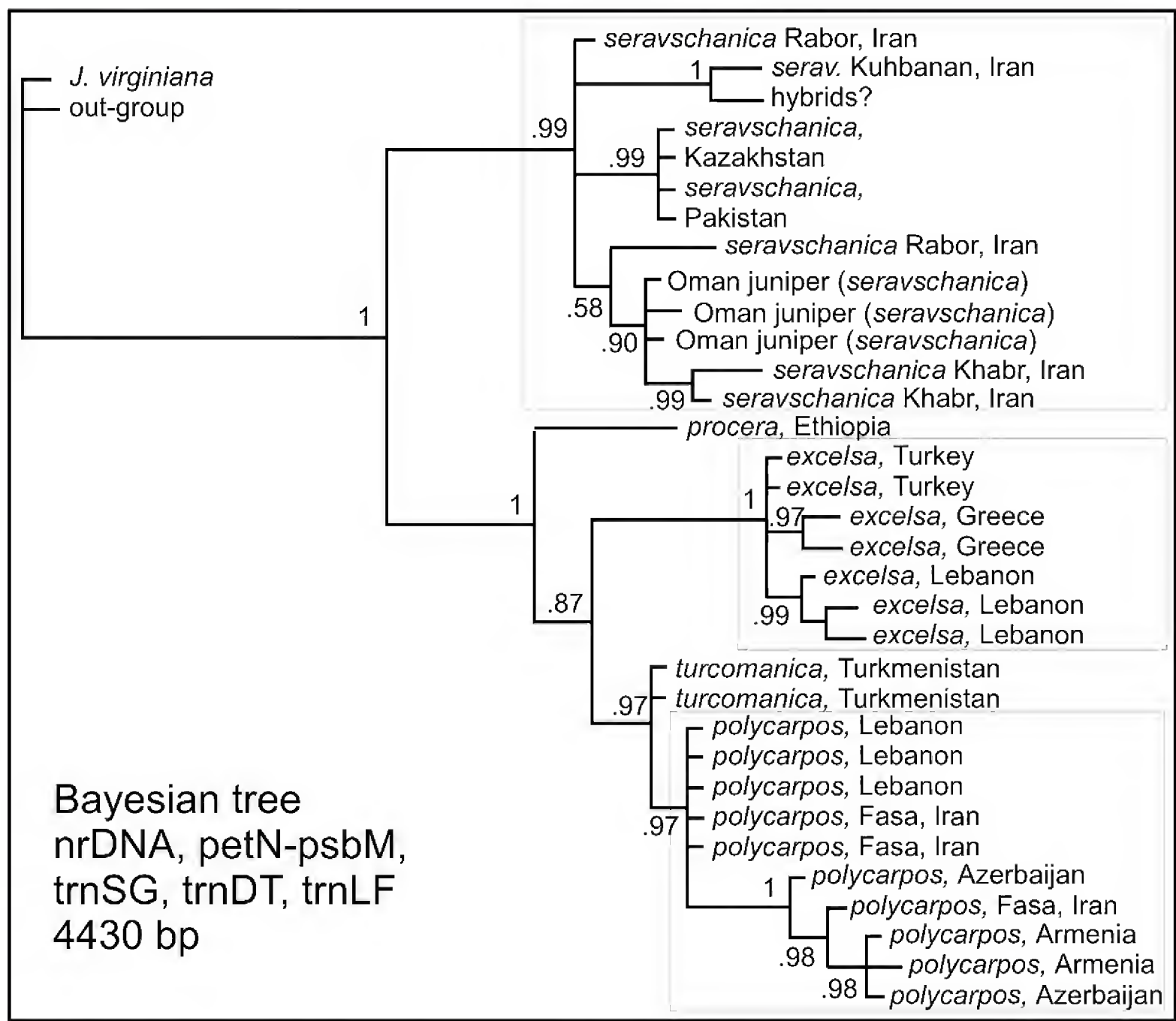


Figure 2. Bayesian tree based on nrDNA (ITS) and four cp regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF (4430 bp). Numbers at the branch points are posterior probabilities. Boxes enclose *J. seravschanica*, *J. excelsa* and *J. polycarpos*. The Oman junipers are grouped with *J. seravschanica*.

Figure 3. *Juniperus seravschanica*, Al Hajar Mtns., Oman. The species is locally common (see Fig. 5).



Figure 4. Leaves and seed cones on *J. seravschanica*. Note the small, new seed cones (inset). Photo taken on 19 Feb. 2014. Pollination appears to have occurred in the winter (Dec. - Feb.).

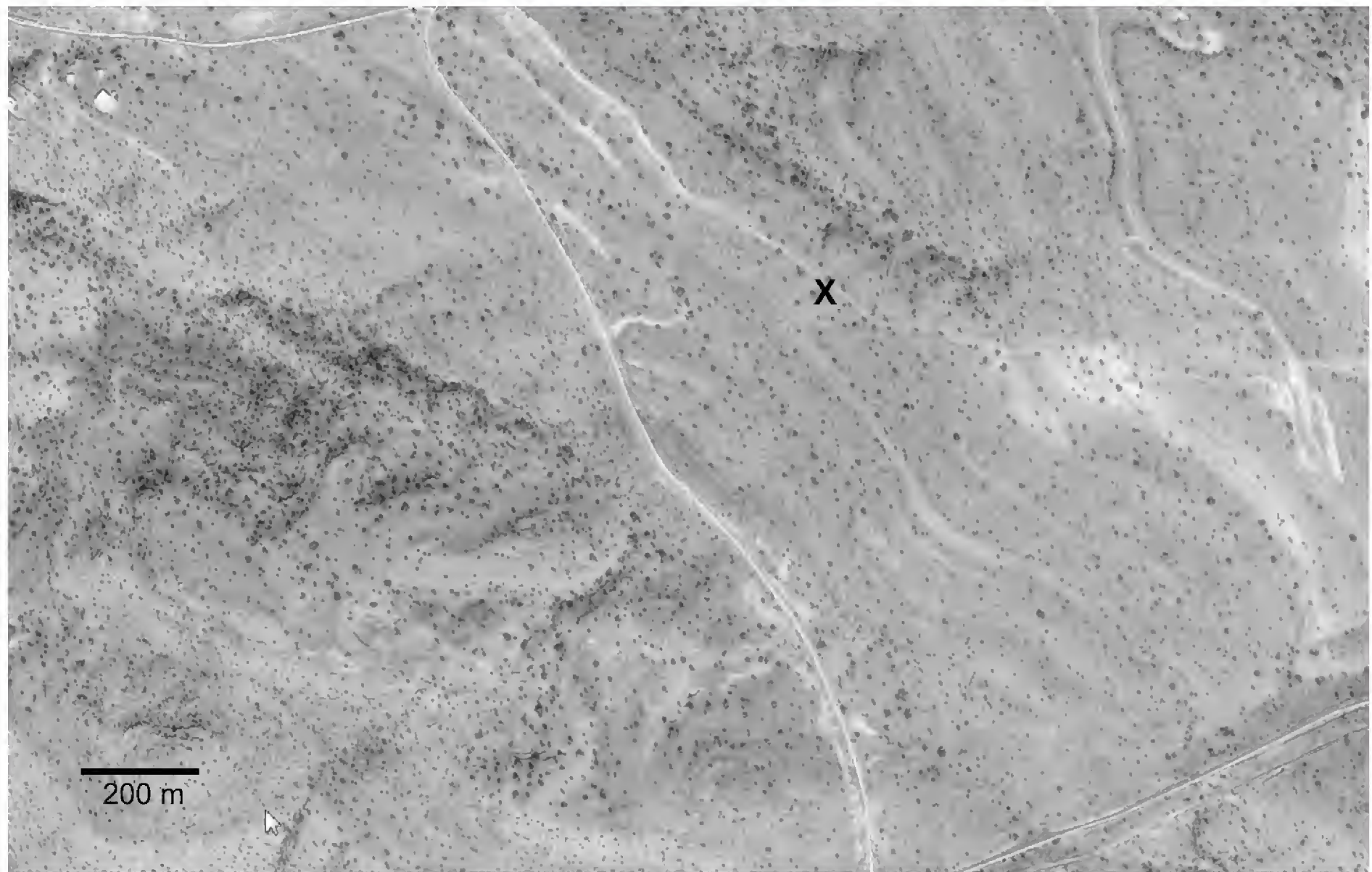


Figure 5. Satellite photo of the population of *J. seravschanica* in the Al Hajar Mtns., sampled for this study. X marks the area where trees were sampled. Nearly all the trees in the photo are *J. seravschanica* (photo courtesy of Google Earth).

The exact distribution of *J. seravschanica* is not known. Plotting specimens from Kew and ON shows (Fig. 6) that all these collections were from an area only about 100 km long. However, as seen in the satellite photo (Fig. 5), the taxon can be rather common in this region. Additional field explorations will be needed to fully map the range of *J. seravschanica* in the Al Hajar Mtns. of Oman.

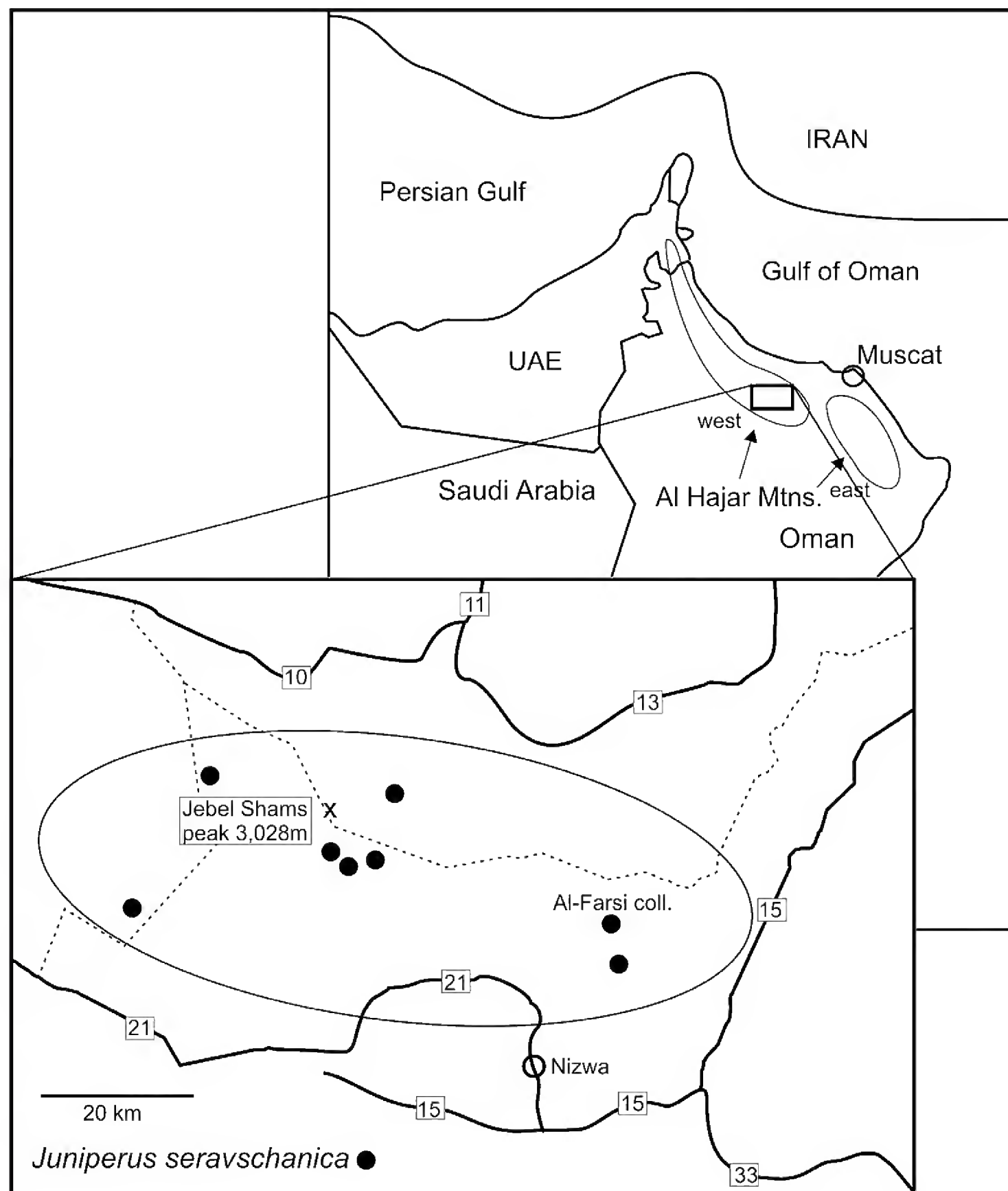


Figure 6. Distribution of *J. seravschanica* mapped from specimens at Kew and ON. The location of the collection (by A. Al-Farsi, 2/2014) for this study is noted.

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Notes on *Lythrum salicaria* L. in Texas and on its distribution on Palo Duro Creek, Randall County, Texas

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ABSTRACT

Purple loosestrife (*Lythrum salicaria* L.) is an invasive, exotic plant in North America. We mapped the distribution of this plant on the terminal 5.7 km of Palo Duro Creek, Randall County, Texas, in 2009. We also searched for it along the margins of other creeks/rivers associated with this section of Palo Duro Creek, including the first 10.5 km of the Prairie Dog Town Fork of the Red River. Further, we collated 16 records from Texas collected specimens located in herbaria, including a Gray County record that has not been previously annotated. Purple loosestrife distribution along Palo Duro Creek is still restricted to the area where it was initially documented in 1975. Published on-line www.phytologia.org *Phytologia* 96(4): 225-234 (Oct 1, 2014). ISSN 030319430

KEY WORDS: *Lythrum*, *L. salicaria*, purple loosestrife, invasive species, wetlands, Palo Duro Creek, Prairie Dog Town Fork of the Red River, Randall County, Texas

Purple loosestrife (*Lythrum salicaria* L.) is a perennial wetland plant that is native to Eurasia (Stuckey 1980). It was first introduced to eastern North America in the late 1700s through shipping and again in the early 1800s as a medicinal herb and ornamental (Stuckey 1980, Malecki et al. 1993, Cox 1999). It occurs in damp floodplains, wet meadows, freshwater marshes, and along open (non-forested) stream and pond margins (Thompson et al. 1987, Cox 1999). It spread rapidly due to its ability to colonize disturbed wetlands, constructed waterways, and irrigation canals (Thompson et al. 1987), tendency to displace/out-compete most native species (e.g., *Typha latifolia* L., Weihe and Neely 1997), prolific seeding (Thompson et al. 1987), lack of suppression by herbivores (Galatowitsch et al. 1999), and popularity with horticulturists and beekeepers (Hayes 1979, Stuckey 1980, Thompson et al. 1987). Where established, it often occurs in monocultures (Weihe and Neely 1997). It may impact the life-cycle (e.g., pollination) of native species (e.g., *L. alatum* Pursh, Brown et al. 2002), alter plant, insect, fish, bird, and mammal communities (Grout et al. 1997, Weihe and Neely 1997, Blossey et al. 2001, Catling 2005, Schooler et al. 2006, 2009), and negatively impact wetland ecosystems (Grout et al. 1997, Blossey et al. 2001, Schooler et al. 2006). In the United States, it is estimated that purple loosestrife collectively cost conservation agencies and landowners \$45 million per year in forage losses, control costs (e.g., chemical, biological, and mechanical treatment), and wetland restoration costs (Malecki et al. 1993, Pimentel 2009, Barbier et al. 2013).

Herbaria records from two Texas locations (Randall and Hardin Counties) are reported in Stuckey's (1980) review of the species distribution (see also Figure 8 in Thompson et al. 1987 and page 432 of Turner et al. 2003). Stuckey's (1980) Hardin County record was first annotated in Flook (1975); it involves a specimen collected near Sour Lake, Texas, on 25 Jun 1971 (Collectors: *Amerson & Watson 540*, Southern Methodist University [SMU] herbarium). The disposition of this specimen is currently unknown – it was likely misidentified, has since been re-determined, and is now filed under a different species name (A. Neill, Botanical Research Institute of Texas). However, if it was correctly identified, it may have involved a population that did not establish, as the plant is not currently thought to be present in southeast Texas (Jason Singhurst, Botanist, Texas Parks and Wildlife Department, pers. comm.). Although the Texas Invasives Database (www.texasinvasives.org) lists no purple loosestrife records, at least two other websites devoted to tracking invasive species (e.g., Early Detection and Distribution Mapping System [www.eddmaps.org] and Discover Life [www.discoverlife.org]) suggest purple loosestrife occurs in Jefferson County. In both cases, this information is linked to a newsletter article (Sorby 1991) that suggests the plant occurs “in the area of Beaumont, Texas.” The newsletter article does not reference its information source, but it is likely referring to the Sour Lake record (Flook 1975), which was collected < 25 km from Beaumont. Unfortunately, information from Sorby's (1971) article was entered into the U.S. Geological Survey's Nonindigenous Aquatic Species database (reference 10097, P. Fuller, personal communication) as a Jefferson County occurrence and subsequently picked up during data ingestion from that website. In addition, Womack and Schuster (1987) report a specimen from a stock pond in Delta County, Texas, that was accessioned by the SMU herbarium.

In July 2007, we observed purple loosestrife (Figure 1) growing in the flood plain of Palo Duro Creek immediately west of Highway 60, Canyon, Randall County, Texas. Herein, we describe a survey to document the extent of, and map, locations of purple loosestrife up- and downstream from this area. The purpose of the mapping effort was to collect information for use in containing, controlling, and eradicating the plant from the site.

MATERIALS AND METHODS

In July, 2007, purple loosestrife was collected from the site where we initially observed it (green polygon in Figure 2) and also photo documented (Figure 1). Collected specimens were shipped to Jason Singhurst, Botanist, Texas Parks and Wildlife Department, for identification. These specimens were cataloged (accession number 064503) into the Baylor University Herbarium, Waco Texas.

As part of a Boy Scouts of America® Eagle Scout service project (for Jason C. Ray), portions of Palo Duro Creek and the Prairie Dog Town Fork of the Red River in Randall County, Texas, were surveyed for purple loosestrife. The survey took place on 18 July 2009. Additionally, we surveyed areas of Spring Draw and Tierra Blanca Creek immediately upstream of their confluences with Palo Duro Creek (35.00229°N -101.91526°W and 35.00208°N -101.90270°W, respectively). The western edge of the survey area was FM 2590 and Palo Duro Creek (Figure 2). The eastern limit of the survey area was where the Prairie Dog Town Fork of the Red River exits the east boundary of Camp Don Harrington, a Boy Scouts of America® property (35.04273°N -101.83544°W; location “B” in Figure 2).

Surveys were conducted when purple loosestrife was in bloom and conspicuous. All surveyors were familiarized with the plant, include blooming and non-blooming forms (non-blooming plants were typically small), as well as species with somewhat similar flower color (e.g., *Polygonum pensylvanicum* L.). *L. californicum* Torr. & Gray, a native species that is somewhat common in playa wetlands in the Texas Panhandle (Haukos and Smith 1997, 2004), is not likely to be confused with purple loosestrife due to stem and leaf color, leaf shape, inflorescence, flower arrangement, and flower abundance (Graham 1975, Haukos and Smith 1997). Surveyors were split into 4 groups of 4-5 individuals (groups consisting of 1 adult surveyor plus Boy Scout surveyors); each group was assigned a section of the survey area to

cover. Permission to access both private and public property for the survey was received by J.C. Ray prior to the survey. Surveyors walked each side of their assigned section of creek/river. All ponds and damp areas near the creek/river beds were also surveyed. Surveyors recorded the location of individual plants or stands/patches of purple loosestrife with a hand-held GPS unit (e.g., Garmin eTrex®) if available, or marked the approximate location of plants on a large-scale paper map. All marked locations (GPS or paper) were transferred to a geospatial database using ArcGIS (ESRI®) so that the coordinates (Appendix A) could be used to locate plants for herbicide treatment. Plants and plant clusters identified by surveyors were verified by W.P. Johnson before individual plant treatment.

In an attempt to gather information on purple loosestrife specimens from our search area and elsewhere in Texas, we searched online databases (e.g., North American Network of Small Herbaria, Consortium of Northern Great Plains Herbaria) using search terms *Lythrum salicaria* and Texas, or by drilling down through their scientific classification and examining each *L. salicaria* record (e.g., Texas A&M University Vascular Plant Specimen List). Additionally, we contacted selected herbaria when references/annotations in literature suggested they might hold Texas specimens and when their records could not be accessed remotely.

RESULTS AND DISCUSSION

We surveyed approximately 19.2 km of creek/river (Figure 3), including approximately 1.1 km of watercourses on Palo Duro Creek Golf Course (all owned by City of Canyon), 1.1 km of Spring Draw (all owned by West Texas A&M University), 0.8 km of Tierra Blanca Creek, 5.7 km of Palo Duro Creek, and 10.5 km of the Prairie Dog Town Fork of the Red River (starting at the confluence of Palo Duro and Tierra Blanca creeks). No purple loosestrife was found on Spring Draw, Tierra Blanca Creek, or the Prairie Dog Town Fork of the Red River.

Purple loosestrife appears to be well established in the portions of Palo Duro Creek between Palo Duro Creek Golf Course and BNSF Railway (Figure 3). Although stands in this area are not monotypic (they are interspersed with *Typha* spp. and *Schoenoplectus* spp.), they could be described as a Class II level of infestation ([mature plants with 10 or more flowering stems per rootstock and clumps sometimes coalescing, forming aggregate masses], Thompson et al. 1987). All other detections within the surveyed area were individual plants. Individual plants and Class II levels of infestations may still be contained, controlled, or eradicated through treatment (Thompson et al. 1987, Mullen 1998).

Although exact locations of specimens acquired by L.C. Higgins (Table 1) are unknown, they include descriptions such as “Palo Duro Creek north of Canyon,” “Palo Duro Creek west of HWY 87,” and “Hunsley Hills Golf Course” (which is now Palo Duro Creek Golf Course), all areas that were covered by our survey. Considering its invasive nature throughout North America (Galatowitsch et al. 1999), it is surprising that the plant, although established, remains relatively localized along the creek after 30 years. Even so, the seed bank may continue to increase without treatment allowing the plant to remain an invasive threat. Numerous small dams along Palo Duro Creek largely prevent downstream flow except in severe flood events and they may be a factor influencing the current distribution of the plant. Much of the area downstream of the infested zone seems suitable for purple loosestrife, especially the open margins and wet meadows associated with the Prairie Dog Town Fork of the Red River. Severe flood events, particularly if coinciding with dropping of seeds or seed germination (newly germinating seeds may float, Thompson et al. 1987) could potentially lead to downstream dispersal.

Searches of herbaria resulted in 16 specimens from Texas (Table 1), 14 of which were from Palo Duro Creek, Canyon, Randall County. The herbarium specimen from Randall County referenced in Stuckey (1980) was not located, but his annotations indicate it was collected by Higgins 9529 on 24 July 1975 on Palo Duro Creek, Canyon, Texas; this was the same date and approximate location as several

other specimens collected by L.C. Higgins (Table 1). The two other records were from Delta (reported by Womack and Schuster 1987) and Gray counties. We were not able to locate the specimen collected in Hardin County (Flook 1975).

CONCLUSIONS

Although purple loosestrife has been documented along Palo Duro Creek in the past (Stuckey 1980), efforts from our survey provide an update on its status. Additionally, we report a herbarium record for Gray County that has not yet been incorporated into reports pertaining to the species distribution in Texas (Turner et al. 2003). Howells (1992) suggested that purple loosestrife is “not problematic” in Texas, and our results suggest the plant has a limited, but established presence in the state. Locations where specimens were collected in Delta, Gray, and Hardin counties warrant additional field investigations, as the current status of purple loosestrife in these areas is unknown.

Mapping efforts from this study are being used in attempts to control the species spread and prevent further seed production/proliferation. Thompson et al. (1987) noted the simple presence of established purple loosestrife, as documented on Palo Duro Creek in this study, suggests it has the potential to become problematic. Aggressive treatment of purple loosestrife has been questioned (Hager and McCoy 1998) due to its impacts to native flora and fauna being potentially overstated (i.e., too few well-conceived research efforts documenting negative impacts, particularly pertaining to impacts on species of concern) relative to the high costs of annual control efforts nationwide (Pimentel 2009); however, these arguments are generally aimed at situations where infestation levels would make it difficult to prevent further spread of the species.

ACKNOWLEDGMENTS

We thank the scouts, troop leaders, and parents of Boy Scouts of America® Troop 31, and R. Johnson, for assistance with the fieldwork associated with this project. We are indebted to the landowners of Spring Draw, Tierra Blanca Creek, Palo Duro Creek, and the Prairie Dog Town Fork of the Red River for allowing us access to their land. We thank the following individuals for physically checking herbarium specimens: A. Neill (SMU collection, Botanical Research Institute of Texas), A. Doran (University of California-Berkeley), S. Rogstad (University of Cincinnati), M. Reed (Texas A&M University-College Station), and W. Holmes (Baylor University). P. Fuller, U.S. Geological Survey, performed queries of the Nonindigenous Aquatic Species database on our behalf. Jason Singhurst assisted with specimen identification. We acknowledge L.C. Higgins for his contributions to herbaria collections. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Table 1. Herbarium specimens of *Lythrum salicaria* collected in Texas.

Date Collected	Catalog Number	Location (County)	Herbarium ¹	Collector, collector ID	Source
24 Jul 1975	UTC00149021	Palo Duro Creek (Randall)	UTC	L.C. Higgins, 9529	SEINet ²
24 Jul 1975	25157	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 9529	R. Kazmaier
24 Jul 1975	00290954 ³	Palo Duro Creek (Randall)	TEX-LL	L.C. Higgins, 9529	Flora of Tex. Database ⁴
24 Jul 1975	365705	Palo Duro Creek (Randall)	NY	L.C. Higgins, 9529	GBIF data portal ⁵
28 Aug 1975	365704	Palo Duro Creek (Randall)	NY	L.C. Higgins, 9753	GBIF data portal ⁵
28 Aug 1975	25458	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 9753	R. Kazmaier
11 Jul 1976	30672	Palo Duro Creek (Randall)	WTS	S. Christiansen, 155	R. Kazmaier
6 Sep 1976	30769	Palo Duro Creek (Randall)	WTS	S. Christiansen, 318	R. Kazmaier
4 Jul 1977	25169	Lake McClellan (Gray)	WTS	L.C. Higgins, 9516	R. Kazmaier
2 Sep 1977	366329	Palo Duro Creek (Randall)	NY	L.C. Higgins, 11366	GBIF data portal ⁵
2 Sep 1977	33795	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 11366	R. Kazmaier
1 Jul 1982	BRIT21435 ³	near Ben Franklin (Delta)	BRIT-SMU	C. Womack, 109	A. Neill
8 Sep 1984	50600	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 14878	R. Kazmaier
30 Oct 1984	50769	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 15086	R. Kazmaier
5 Sep 1986	365795	Palo Duro Creek (Randall)	NY	L.C. Higgins, 16998	GBIF data portal ⁵
5 Sept 1986	54409	Palo Duro Creek (Randall)	WTS	L.C. Higgins, 16998	R. Kazmaier

¹UTC = Utah State University; WTS = West Texas A&M University; TEX-LL = University of Texas-Austin; NY = New York Botanical Garden; BRIT-SMU = Southern Methodist University/Botanical Research Institute of Texas

²swbiodiversity.org/seinet <accessed 24 Jan 2014>

³barcode number

⁴Plant Resources Center, www.biosci.utexas.edu/prc/Tex.html <accessed 23 Jan 2014>

⁵www.gbif.org <accessed 24 Jan 2014> and sciweb.nybg.org <accessed 6 Feb 2014>



Figure 1. Photos: (A) patch of *Lythrum salicaria* (the purple flowering plant); (B) a robust clump of *L. salicaria*; and (C) blooms of *L. salicaria*. The plants in these photos were all located about 230 meters west southwest of the Palo Duro Creek – Hwy 60 intersection in Canyon, Randall, County, Texas (approximately 34.99648° -101.92128°). Photos taken 27 July 2007 by W.P. Johnson.

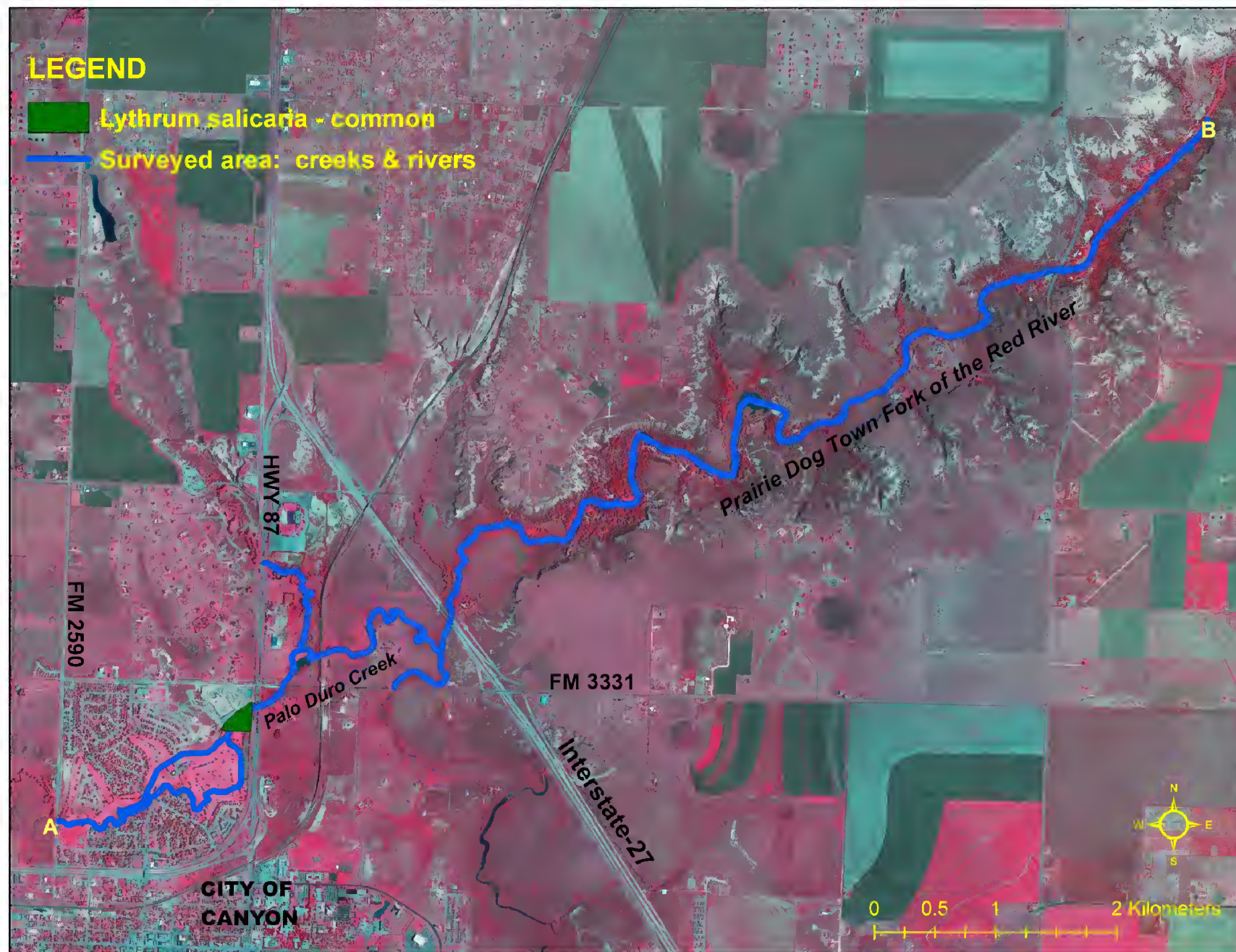


Figure 2. Creek and river margins surveyed for purple loosestrife (A = western extent of surveyed area; B = eastern extent of surveyed area) on 18 July 2009, Randall County, Texas. All highlighted sections (blue) of creek/river were searched. The area marked as “*Lythrum salicaria*-common” captures the area of the pictures in Figure 1. Background image 17 December 2004, courtesy of National Agriculture Imagery Program, Farm Services Agency, U.S. Department of Agriculture, Salt Lake City, Utah.

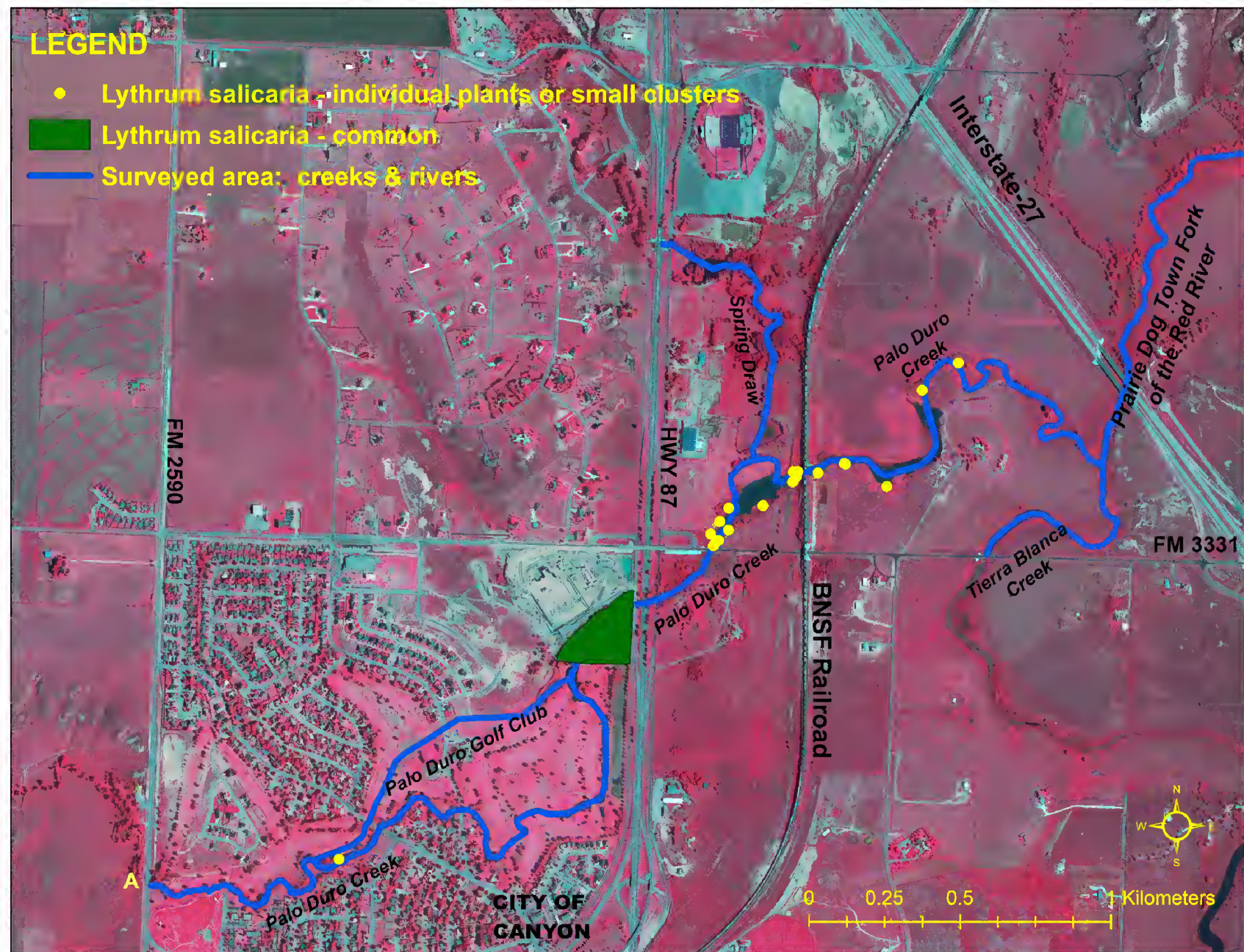


Figure 3. Distribution of purple loosestrife along Palo Duro Creek in Randall County, Texas, on 18 July 2009. All highlighted (blue) sections of creeks/rivers were searched (A = west extent of surveyed area). The area marked as “*Lythrum salicaria*-common” captures the area of the pictures in Figure 1. Background image 17 December 2004, courtesy of National Agriculture Imagery Program, Farm Services Agency, U.S. Department of Agriculture, Salt Lake City, Utah.

Appendix A. Coordinates (decimal degrees) of individual *Lythrum salicaria* plants and small clumps/clusters located by the 18 July 2009 mapping effort (see Figure 3). Plants in the area immediately west of the Palo Duro Creek – Hwy 60 intersection (the area represented by the green polygon in Figure 3) are not included. Coordinates are in Geographic Coordinate System: GCS North American 1983, Datum: D North American 1983.

Latitude	Longitude
35.00221°	-101.91207°
35.00217°	-101.91199°
35.00152°	-101.91049°
35.00447°	-101.90935°
35.00531°	-101.90808°
34.98994°	-101.92999°
34.99972°	-101.91667°
35.00009°	-101.91617°
35.00088°	-101.91495°
35.00158°	-101.91393°
35.00176°	-101.91384°
35.00187°	-101.91299°
35.00187°	-101.91375°
35.00188°	-101.91396°
35.00036°	-101.91656°
35.00076°	-101.91622°
34.99995°	-101.91690°
34.99958°	-101.91678°
34.99976°	-101.91660°

Texas taxa of the genus *Berlandiera* (Asteraceae: Heliantheae)

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ABSTRACT

The state of Texas is recognized as having two species of *Berlandiera*, a widespread *B. lyrata* Benth., represented by the newly proposed var. **purpurea** B.L. Turner, **var. nova.**, and a more localized species of high elevation, *B. macvaughii* B.L. Turner, **sp. nov.**, of trans-Pecos, Texas. Photographs of the types are provided, along with maps showing their distribution.

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KEY WORDS: Asteraceae, *Berlandiera*, *B. lyrata*, *B. macvaughii*, Texas, trans-Pecos, Sierra Madera, McKittrick Canyon, Glass Mts.

BERLANDIERA LYRATA Benth., Pl. Hartw. 17. 1839.

Two geomorphological population systems are recognized within this species; the typical var. **lyrata** is confined to Mexico, but var. **purpurea** extends into northwestern Mexico from the southwestern USA.

- 1. Disc florets yellow; se Chi, Dur, Agu, Zac, San, n Jal.....var. **lyrata**
- 1. Disc florets purple or brownish-purple; Son, n Chi, Coa, USA..... var. **purpurea**

var. **purpurea** B.L. Turner, **var. nov.**

Berlandiera incisa Torr. & Gray

Resembling *B. lyrata* var. **lyrata** but the disc florets purple to brownish-purple (vs yellow), the involucres somewhat smaller (12-15 mm wide vs 15-20 mm), and the achenes mostly 5-6 mm long (vs 4-5); chromosome number, n = 15 pairs.

TYPE: USA. TEXAS: Jeff Davis Co.: Wild Rose Pass, on Hwy 17, ca 22 mi S of Balmorhea city limits, 16 May 1989, *Lindsey Woodruff* 296 [with Mark Mayfield] (Holotype: TEX). **Fig. 1.**

ADDITIONAL SELECTED SPECIMENS EXAMINED: USA. NEW MEXICO: Guadalupe Co.: 25.5 mi SE of Santa Rosa, 10 Aug 1988, *Turner* 15831 (TEX).

TEXAS: Brewster Co.: Alpine, vacant lot across from SRSC campus, 8 Mar 1988, *Turner* 15801 (TEX). **Culberson Co.:** NE part of County, where hwy 285 crosses into Reeves Co., 6 Apr 2001, *Turner* 21-224 (TEX). **King Co.:** ca 2 mi S of Guthrie, 11 Sep 2006, *Turner* 26-49 (TEX). **Martin Co.:** 4.2 mi W of Stanton, 16 Apr 2000, *Turner* 20-77 (TEX). **Menard Co.:** north-central part of County where hwy 83 crosses into Concho Co., 1 Jun 2000, *Turner* 20-361 (TEX). **Reeves Co.:** In center of Balmorhea, waste places, 23 Aug 1999, *Turner* 99-638 (TEX). **Map 1.**

Most of the specimens of Texas represented on my dot maps were also examined and identified accordingly.

The novelty is named for its consistently purple or purplish disc florets (vs yellow, as occurs in var. **lyrata**).

BERLANDIERA MACVAUGHII B.L. Turner, **sp. nov.**

Perennial herbs 15-35 cm high, the stems arising from enlarged woody or lignescent roots. **Leaves** mostly basal, 10-18 cm long, 1.5-4.0 cm wide; petioles 2-7 cm long; blades ovate to linear-oblongate, passing into the petioles, scarcely lobed, if at all, the stem leaves mostly absent (except in occasional putative hybrids with **B. lyrata**), pinnately veined, sparsely pubescent above, more so beneath, the margins crenulate. **Heads** 3-5 cm across the extended rays, borne 1 or 2 to a peduncle, the latter 5-25 cm long. **Involucres** 4-5 seriate, scarcely gradate, the inner series broadly ovate, 10-15 mm long and as wide, their apices to some extent broadly acute to narrowly obtuse, not rounded. **Pales** linear-oblongate, 5-8 mm long, 1-2 mm wide, their apices rounded. **Ray florets** 8, fertile; ligules 10-15 mm long, 3-5 mm wide, yellow on both surfaces, the lower surfaces with 8 or more weakly defined, yellowish veins (as opposed to the fewer, dark purple, well defined veins on the under-surfaces of ligules in **B. lyrata**), irregularly 3-lobed at apex. **Achenes** broadly ovate to cordate, 5-6 mm long, 3-5 mm wide, epappose; dorsal surfaces glabrous; ventral surfaces pubescent, their margins mostly w/o attached pales at maturity. **Disc florets** staminate, numerous; corollas yellow, 4-5 mm long; tube ca 0.5 mm long; throat ca 3 mm long, glabrous; lobes ca 0.5 mm long, pubescent.

TYPE: **USA. TEXAS: Pecos Co.:** "Rocky (limestone) slopes, main canyon on northeast side of Sierra Madera, about 25 miles south of Fort Stockton." ca 1300 m, 25 May 1949, *Rogers McVaugh 10649* (Holotype: TEX; isotype: SRSC). **Fig. 2.**

ADDITIONAL SPECIMENS EXAMINED: **NEW MEXICO. Eddy Co.:** "SW portion of Dark Canyon, near creek bed, reddish sandy loam soil; juniper-ponderosa woodland." 1 Sep 1965, *Powell & Sikes* (SRSC, TEX). **TEXAS. Brewster Co.:** "Rare near top of Baldy Peak, Glass Mts." 13 Jul 1940, *Warnock 255* (TEX). **Culberson Co. [Guadalupe Mts]:** "South fork of McKittrick Canyon." 27 Sep 1962, *Correll & Correll 26043* (LL); "South Fork of McKittrick Canyon, 21 Jun 1964, *Correll & Hanson 29828* (LL); "South McKittrick Canyon, 12 Jul 1948, *Hinckley 4494* (SRSC); "grassy slopes in the Bowl," 21 Jun 1958, *Johnston 3189* (SRSC); "Pine Top Mt.," 15 Sep 1958, *Turner & Warnock 176* (SRSC); "lower McKittrick Canyon," 30 Apr 1961, *Warnock 18426* (SRSC, TEX); "South McKittrick Canyon," 26 Apr 1962, *Warnock 18487* (SRSC); "Upper Pine Spring Canyon, 3 Apr 1947, *Warnock & Harvill 5523* (SRSC); "South McKittrick Canyon, 18 May 1958, *Warnock & Johnston 16459* (SRSC); "south slope of Pine Top Mt., 28-29 Jul 1952, *Webster 4576* (SRSC); South McKittrick Canyon, 10 Jun 1971, *Weston 79* (SRSC); "Rocky Bluffs near Pine Springs." 15 Aug 1916, *Young s. n.* (SRSC, TEX). **Map 2.**

As might be noted, **B. macvaughii** is abundant in The Guadalupe Mts. of Culberson County. It is apparently rare in the higher elevations of the Glass and Sierra Madera Mts., to judge from the few collections assembled to date from the two sites. So far as known, the widespread, commonly encountered, **B. lyrata** does not grow with or near **B. macvaughii**.

The collection *Correll & Correll 26043* from McKittrick Canyon has the general habit and stem-leaves of **B. lyrata** var. **purpurea**, but it is a larger plant with larger, more pinnatifid, basal leaves, having yellow disc florets and achenes with peripheral pales attached. This may prove to be a hybrid (perhaps ancestral) between the two taxa concerned, but so far as known the two species do not occur together.

Pinkava (2006), presumably, would position most of the specimens cited here in his broad concept of **B. lyrata**, noting that "Exceptional specimens that are scapiform (sometimes monocephalic) with mostly undivided leaves and with wartlike hairs on peduncles occur at higher elevations (south-central New Mexico, trans-Pecos Texas, and Nuevo Leon). They have yellow disc corollas, as do most collections from Chihuahua, Durango, Nuevo Leon and Tamaulipas." The material from New Mexico

and trans-Pecos, Texas, referred to by Pinkava I would assign to **B. macvaughii**; those from Nuevo Leon I treat as **B. basalaris** B.L. Turner (in prep).

In my treatment of **Berlandiera** for Mexico (in prep) I intend to recognize six taxa, including **B. lyrata** var. **purpurea**.

It is a pleasure to name the novelty for the late Rogers McVaugh (1909-2009), collector of the Type and well-known student of the trans-Pecos flora. As indicated, Rogers lived to be 100 years old, setting standards for those of my ilk, just ready to turn 90. I first met him in Alpine, Texas in 1947, while a pre-law student at Sul Ross State College, having taken a few botany classes on the side to satisfy my interest in natural history. My field trip up Cathedral Mt. with McVaugh and Warnock in the late 1940s largely convinced me that I had rather be a botanist than a lawyer. McVaugh remained one of my closest friends throughout the remainder of his life, always a guest at my house in Austin, Texas, while passing through on the way from Michigan to his first love, botanically speaking, Mexico.

ACKNOWLEDGEMENTS

Thanks to my Academic son, Mike Powell, for helpful comments and calling to my attention the plants of **B. macvaughii** on file at SRSC, these cited above; and to my editorial colleague, Jana Kos, for meaningful input. The dot maps are largely based upon Turner et al. (2003), specimens on file at LL-TEX, SRSC and from USDA records available on the web.

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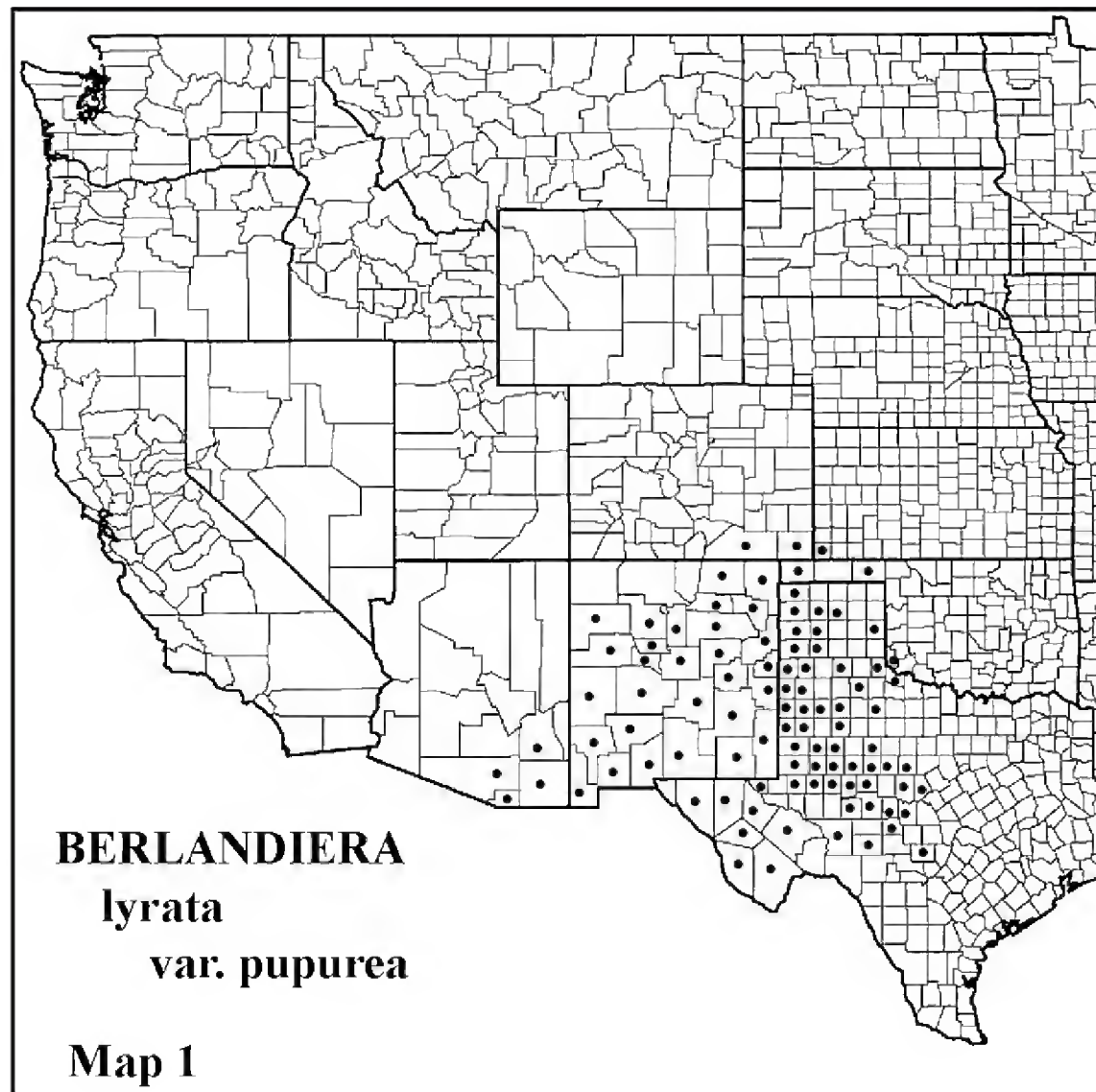
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Turner, B.L., et al. 2003. Atlas of the Vascular Plants of Texas 1: 70. [SIDA, Bot. Misc. 24]



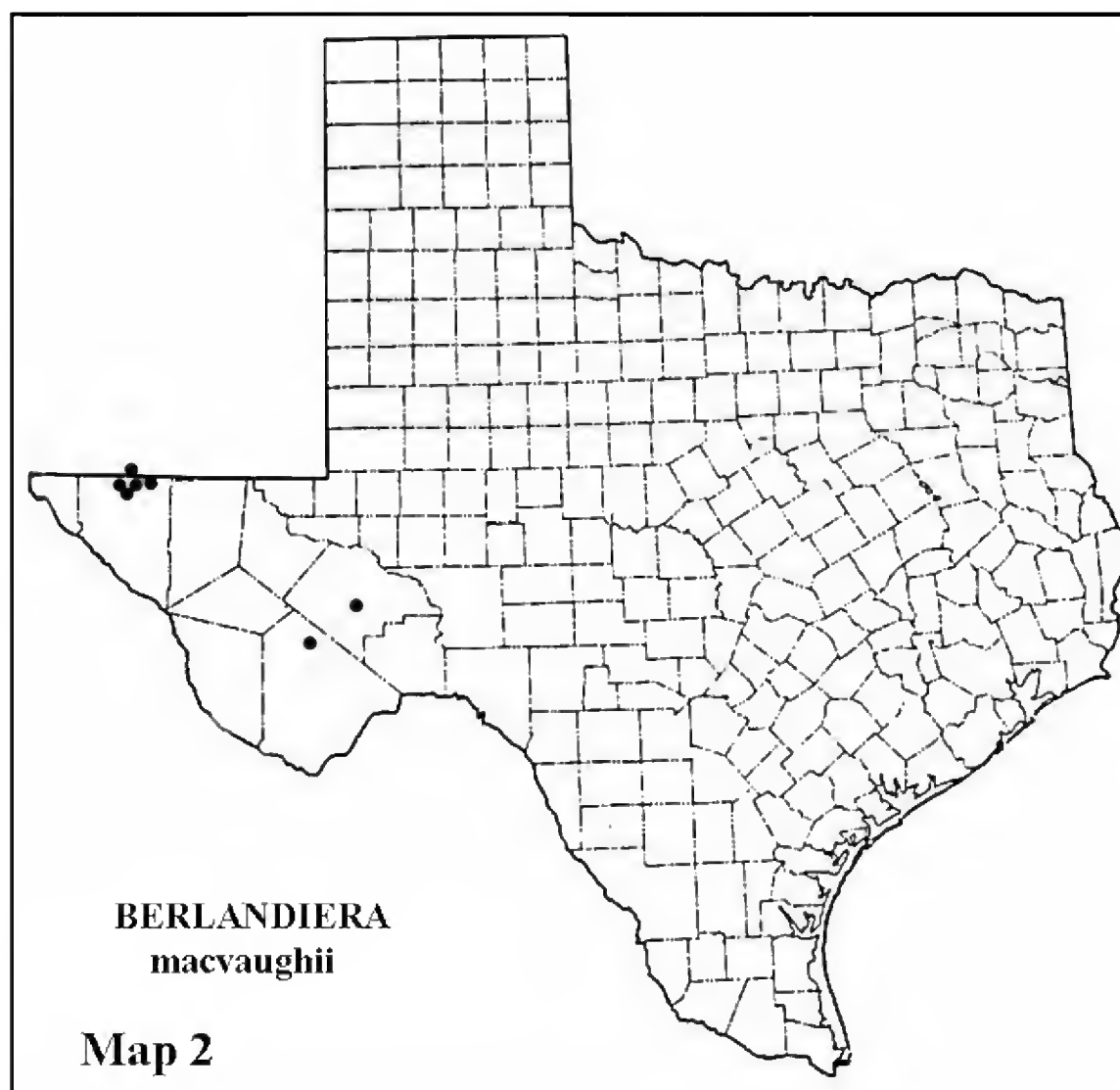
Fig. 1. Holotype of *Berlandiera lyrata* var. *purpurea* (TEX).



Fig. 2. Holotype of *Berlandiera macvaughii* (TEX).



Map 1. Distribution of *Berlandiera lyrata* in the USA.



Map 2. Distribution of *Berlandiera macvaughii*.

**A closer look at *Asclepias engelmanniana* Woodson and *Asclepias rusbyi* (Vail) Woodson
(Apocynaceae, Asclepiadoideae)**

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ABSTRACT

A closer look is taken at the anther wings of *Asclepias engelmanniana* and *A. rusbyi* and important differences are described. It is postulated that they are better diagnostic characters than the rudimentary horns of both taxa, and that the two taxa should be retained as distinct species. Photos and diagrams are provided to illustrate the thesis. Published on-line www.phytologia.org *Phytologia* 96(4): 241-246 (Oct 1, 2014). ISSN 030319430

KEY WORDS *Asclepias engelmanniana*, *Asclepias rusbyi*, Apocynaceae, Asclepiadoideae, anther wings, horns.

Ever since Elliott (1817) published the genus *Acerates* (Greek for ‘without horns’), the presence or absence, and the size when present, of the coronal horn has been considered an important taxonomic character by authors working on Apocynaceae, subfamily Asclepiadoideae. Yet Elliott himself had doubts about its importance, and he was not alone in this regard. Greene (1897) deplored the “fanciful and exaggerated importance...given to that diminutive organ.” Yet the suggestion of the importance of the horns persists to this day, even though *Acerates* has long been considered a synonym of *Asclepias*. After Vail (1898) distinguished *Acerates rusbyi* Vail from *A. auriculata* Engelm. ex Torr. (1859), there has been contention over the suitability of such separation. Vail did not mention the horn in *A. auriculata*, but she noted that the anther wings were “incurved over the anthers at the summit.” With *A. rusbyi* she did cite the horn, but also noted that the anther wings were merely “salient and notched near the base.” However, she did not find these differences to be taxonomically significant. Later, Woodson (1941) incorporated *Acerates* Elliott into *Asclepias* L. and replaced *Acerates auriculata* with the new name, *Asclepias engelmanniana* Woodson, because of the prior publication (1819) of *Asclepias auriculata* Kunth. Kearney (1949) subsequently reduced *A. rusbyi* to a variety, *A. engelmanniana* var. *rusbyi* (Vail) Kearney, citing only its horn to distinguish it from var. *engelmanniana*. A few years later, Woodson (1954) raised it back up to species rank as *A. rusbyi* (Vail) Woodson, citing both the horn and the anther wings; finally Sundell (1990) upheld its varietal status, again citing only the horn. In the two cases arguing for varietal status, the rudimentary and highly-variable horn was given major taxonomic significance, all the while a much more reliable character mentioned by Vail and used by Woodson was given no notice at all, i.e. the anther wings or guide rails.

OBSERVATIONS & CONCLUSIONS

The anther wings of *A. engelmanniana* are unique among all species of *Asclepias* in that they arch in such a way as to position the corpuscula of the pollinaria directly on top of the stigma head, rather than along its sides as in all of the other species. Yet Sundell’s (1993) observation of this character was limited to “anther wings 2-2.4 mm long,” while he described the horn at length (50 words). By contrast the anther wings of *A. rusbyi* are more conventional, with only a slight curve, not an arch, and the corpuscula are positioned along the sides of the stigma head. In most species of *Asclepias*, access to the anther wings is at their base, which is often flared to permit the entrance of a leg or bristle of a pollinator moving upwards. In the case of the arched anther wings of *A. engelmanniana*, there may be a flare at the base of the wings, but, due to the arch, this would be of little value to its pollination. Consequently, there is also an additional flaring where the anther wings arch that allows easy access to the corpuscula by a lateral

rather than a vertical movement of the insect's leg. These arched anther wings are a diagnostic character for *A. engelmanniana*, and such are lacking in *A. rusbyi*. Are they important enough to distinguish two taxa? In my view they are and I would maintain *A. rusbyi* as a good species.

Because it is risky to base a species on a single character, no matter how important it may seem, there are yet other characters that can be used to separate the two. In *A. engelmanniana* the column is shorter than in *A. rusbyi* (Vail also noted this) and the gynostegium appears to sit directly upon the corolla. In *A. engelmanniana* the hoods are flattened and adpressed to the anthers, while in *A. rusbyi* the hoods are narrower and somewhat spreading, i.e. they are held farther from the anthers with a space between them. The nectar reservoirs are positioned lower and are deeper in *A. engelmanniana* than in *A. rusbyi*. Finally the horn is positioned lower in the hood in *A. engelmanniana* than it is in *A. rusbyi*. In the latter the horn is often visible. The pollinaria of both species are quite similar. I also note that, due to the similarity of these two species, hybrids should be expected where their ranges overlap. I have seen hybrids between *A. syriaca* L. and *A. speciosa* Torr. in central KS, and those two species are more distinct than the two discussed here. I have also reported on a hybrid between *A. syriaca* and *A. purpurascens* L. in central MO (Rintz 2014), and those two species are also quite distinct.

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I express my gratitude to Ken Heil at San Juan College, NM, without whose assistance I would not have found that wonderful population of *Asclepias rusbyi* in Navajo Co., AZ; and to Roy Gereau and W. D. Stevens for reviewing the manuscript and making many valuable suggestions.

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Figure 1. Two flowers compared: *Asclepias rusbyi* and *Asclepias engelmanniana*.



Figure 2. *Asclepias engelmanniana* flower from Cimarron Co., OK. Note the additional flaring of the anther wings along the arch, besides the flaring at the base.

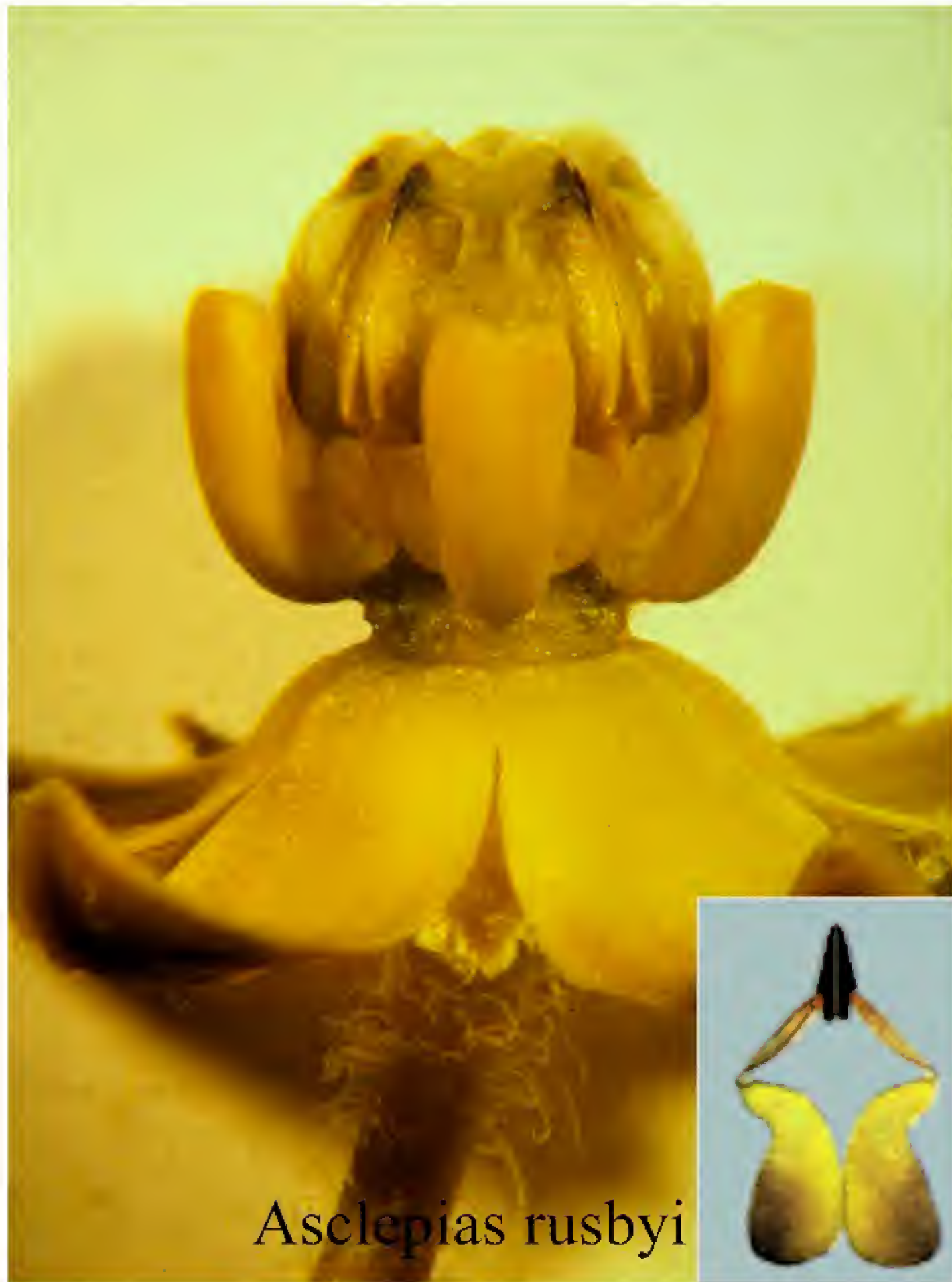


Figure 3. *Asclepias rusbyi* flower from Navajo Co., AZ. Inset shows the pollinarium.



Figure 4. *Asclepias engelmanniana* flower from Armstrong Co., TX. Note the lack of flaring at the base of the anther wings and the deeper notch at the apex of each hood.

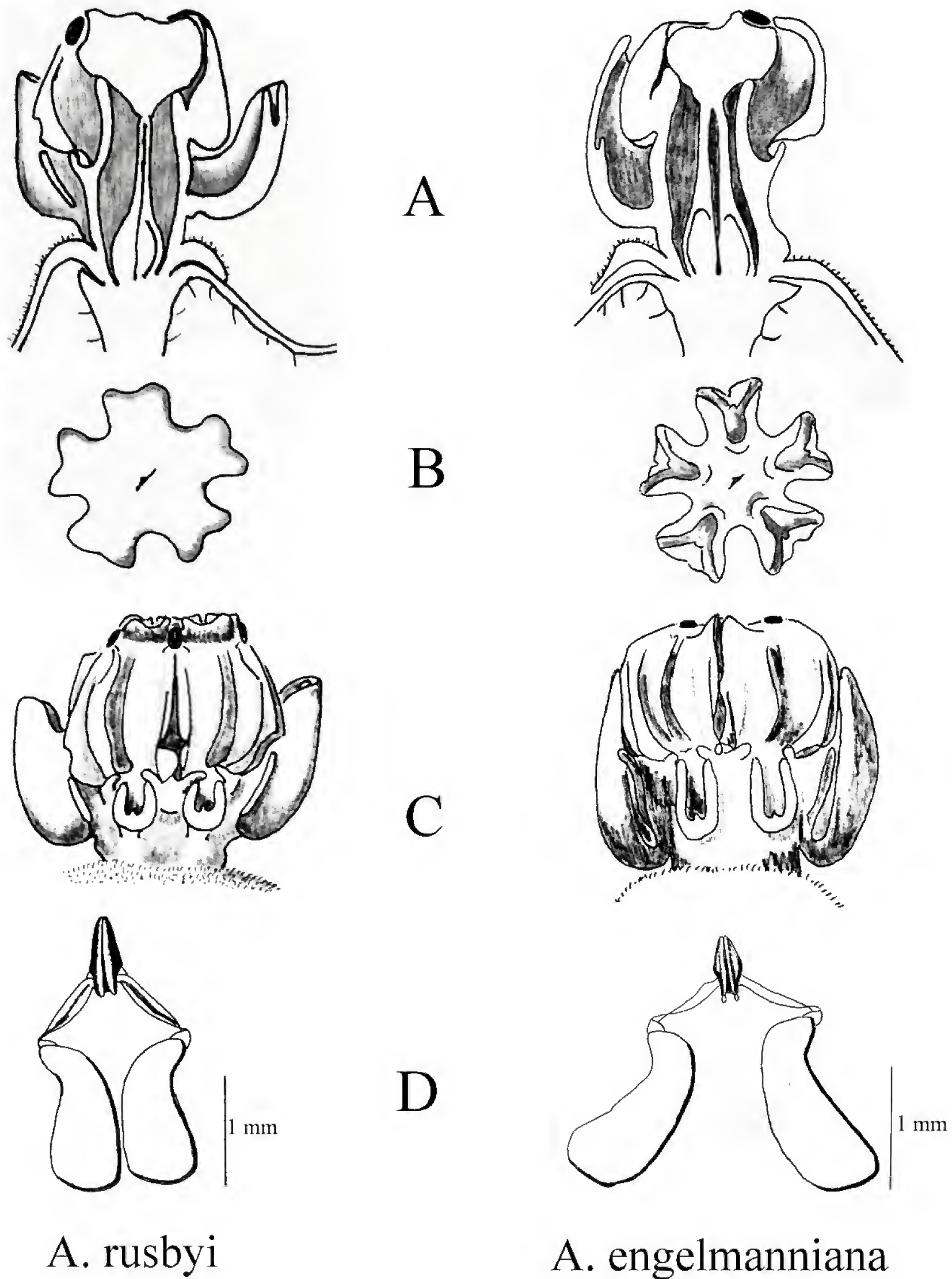


Figure 5. *Asclepias engelmanniana* and *A. rusbyi* compared. A. Radial sections. B. Stigmas seen from above; note the glandular depressions for secretion of the corpuscula and translators in the *A. engelmanniana* stigma. C. Gynostegia with two hoods removed as seen from the side. D. Pollinaria.

**Geographic variation in *J. phoenicea* var. *phoenicea* from throughout its range:
Analysis of nrDNA and the petN-PsbM cp region.**

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ABSTRACT

Juniperus phoenicea (var. *phoenicea*) was examined from five populations throughout its range by sequencing nrDNA and the petN-psbM cp region. In addition, data from seventeen populations of *J. turbinata* (*J. phoenicea* var. *turbinata*) were included in a Bayesian analysis. All populations of *J. phoenicea* were found in a well-supported clade, separate from *J. turbinata*. Little infra-specific variation was found in *J. phoenicea*, with only one substitution present in France, one in Zaragoza and one in El Penon populations. The divergence of the El Penon correlates with a previous report of differences in the leaf volatile oils. Published on-line www.phytologia.org *Phytologia* 96(4): 247-251 (Oct. 1, 2014).

KEY WORDS: *Juniperus phoenicea* var. *phoenicea*, *Cupressaceae*, nrDNA, petN-psbM, geographic variation, *J. turbinata*.

Adams et al. (2013) analyzed nrDNA and petN-psbM sequences for *J. phoenicea* L. (*sensu stricto*) from throughout the Mediterranean region (Fig. 1). They found *J. phoenicea* var. (or subsp.) *phoenicea* to be restricted to Spain and France, whereas *J. phoenicea* var. *turbinata* (Guss.) Parl. (*J. turbinata* Guss.) was widely distributed from the Canary Islands to the Sinai. No differentiation was found between the typical Mediterranean and Canary Island populations, offering no support for the recognition of *J. phoenicea* subsp. *canariensis* (Guyot) Rivas-Martínez (Fig. 1). *Juniperus turbinata* is widespread, ranging from Madeira - Canary Islands to the Sinai with few DNA differences among most populations. However, some populations (Grazalema, Madeira, Sinai, central Italy) displayed (Fig. 1) moderate amounts of divergence (3-4 mutations).

In a broad phylogenetic study of *Juniperus*, Adams and Schwarzbach (2013) found that *J. phoenicea* was not part of a clade of serrate-leaf junipers occurring in the western hemisphere, leading them to denote *J. phoenicea* as a 'pseudoserrate' juniper. In addition, they found *J. p.* var. *phoenicea* and var. *turbinata* to be as different in their DNA sequences as several other recognized species of *Juniperus*. Based on these data, they recognized *J. turbinata* Guss., as had been proposed by Lebreton and Pérez de Paz (2001) based largely on the concentration of prodelphinidin, a polymeric tannin. The prodelphinidin

data indicated that *J. p. var. phoenicea* was confined to the Iberian Peninsula, while var. *turbinata* occurred throughout the Mediterranean region. Lebreton and Pérez de Paz (2001) found a clear separation between *J. phoenicea* (Spain and France) and all other populations examined (*J. turbinata*).

Adams, Altarejos and Arista, 2014) examined the volatile leaf oil of *J. phoenicea* (*sensu stricto*) from throughout its range (Spain and France). They reported the composition of the volatile leaf oils of four of the five populations varied very little except for a chemotype (one tree) in the Zaragoza population that was high in cedrol and other cedarwood terpenoids. However, all five trees sampled in the Grazalema population had the cedarwood chemotype and were high in cedrol. The oil from the high cedrol plants at Grazalema seems quite different due to the presence of cedarwood oil components, but the oil is actually not very different, if one removes the heartwood terpenoids and re-normalizes the remaining terpenoids (Table 1, Adams et al., 2014).

It is of interest to examine variation in DNA sequence data from the same populations (Adams et al., 2014) in France and Spain.

The purpose of the present study is to present analyses of nrDNA and petN-psbM sequences from populations of *J. phoenicea* var. *phoenicea* throughout its range.

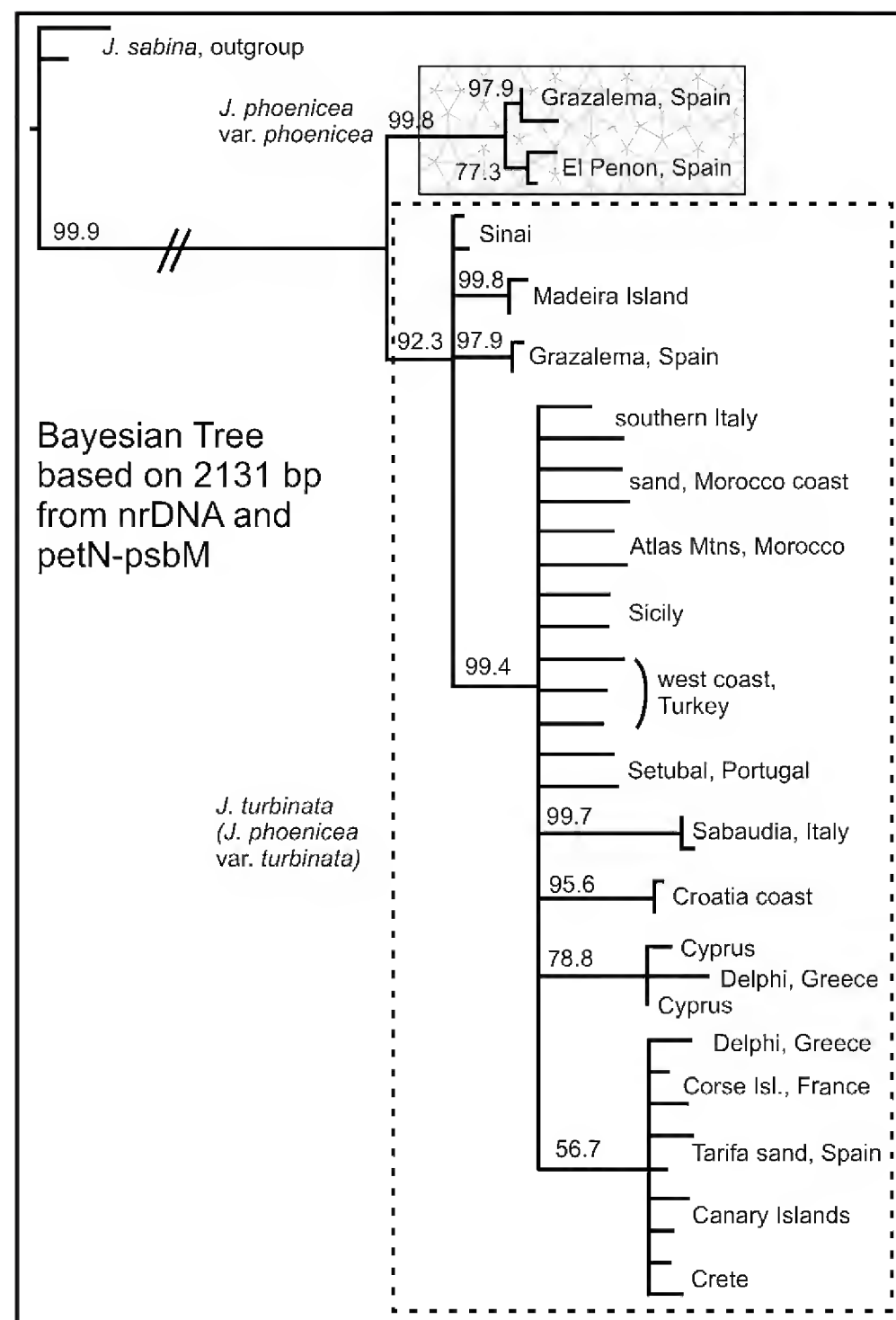


Figure 1. Bayesian tree of *J. phoenicea* and *J. turbinata* (*J. p. var. turbinata*) from throughout the species ranges. (from Adams et al., 2013).

MATERIALS AND METHODS

Figure 2 shows the distributions of *J. phoenicea* var. *phoenicea* and populations sampled in this study.

Specimens used in this study: ***J. p. var. phoenicea*:**

France, Narbonne, near St. Pierre sur Mer, 43° 10' 0.2" N, 3° 09' 57.6" E, 23 m, *J. Altarejos 1-5*, Baylor specs. Adams 14123-14127.

Andorra, Coll de Jou near Sant Julià de Lòria, 42° 26' 56.8" N, 1° 28' 04.6" E, 1426 m, *J. Altarejos 6-10*, Baylor specs. Adams 14128-14132.

Spain, Zaragoza, Montes de la Retuerta de Pina W of Bujaraloz, 41° 28' 59"N, 0° 19' 31.2"W, 317 m, *J. Altarejos 11-15*, Baylor specs. Adams 14133-14137.

Spain, El Peñón, approx. 25 km west of Guadahortuna, 37° 35' 38" N, 3° 31' 22" W, 760 m, Adams 7077-7079,

Spain, Cádiz, Sierra de Grazalema, 36° 47' 51.5" N, 5°24' 43.7"W, 835 m; *M. Arista 1-5*, Baylor specs. Adams 13813-13817.

Locations of populations of *J. turbinata* are listed in Adams et al. (2014). Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20°C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl_2 according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>) and the MAFFT alignment program. Analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion.

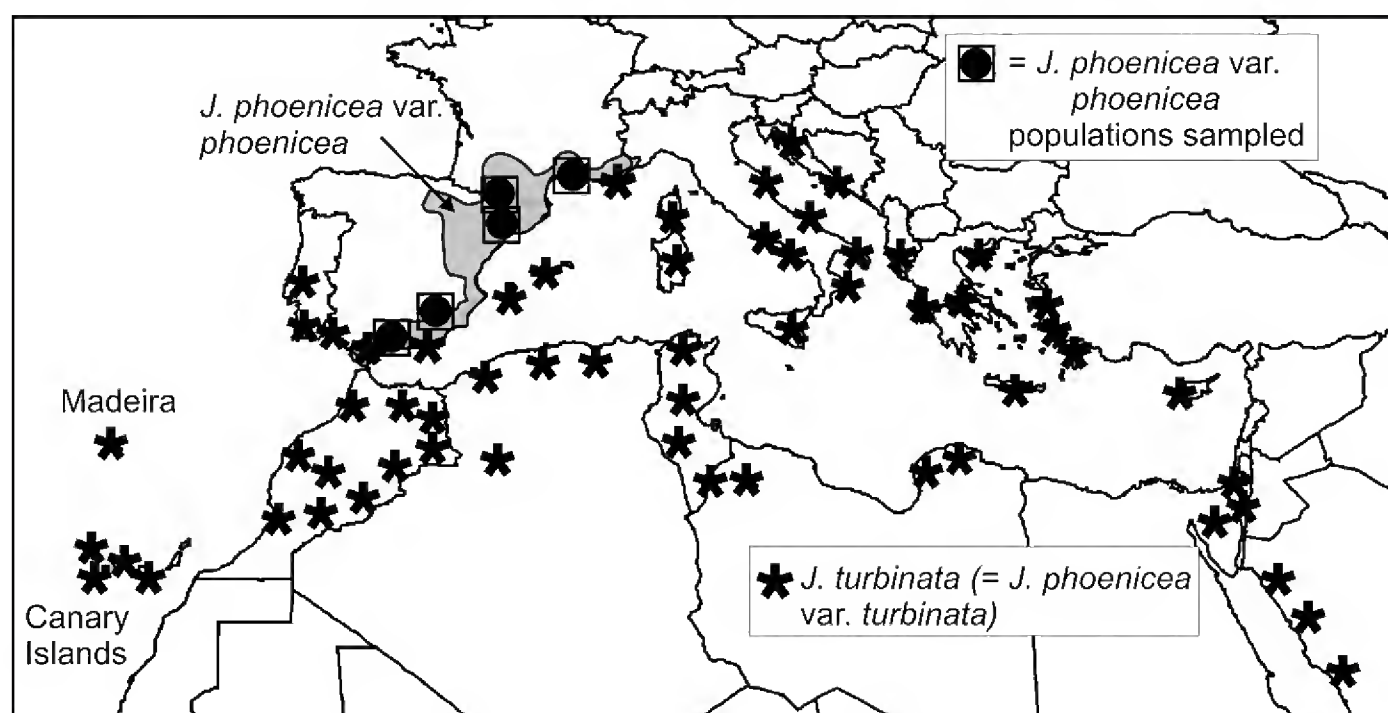


Figure 2. Distributions of *J. phoenicea* and *J. turbinata* (adapted from Adams, 2014; Lebreton and Pérez de Paz, 2001). Gray shaded area shows the general distribution of *J. phoenicea* (var. *phoenicea*) and solid circles inside squares show the five populations of *J. phoenicea* sampled in the present study.

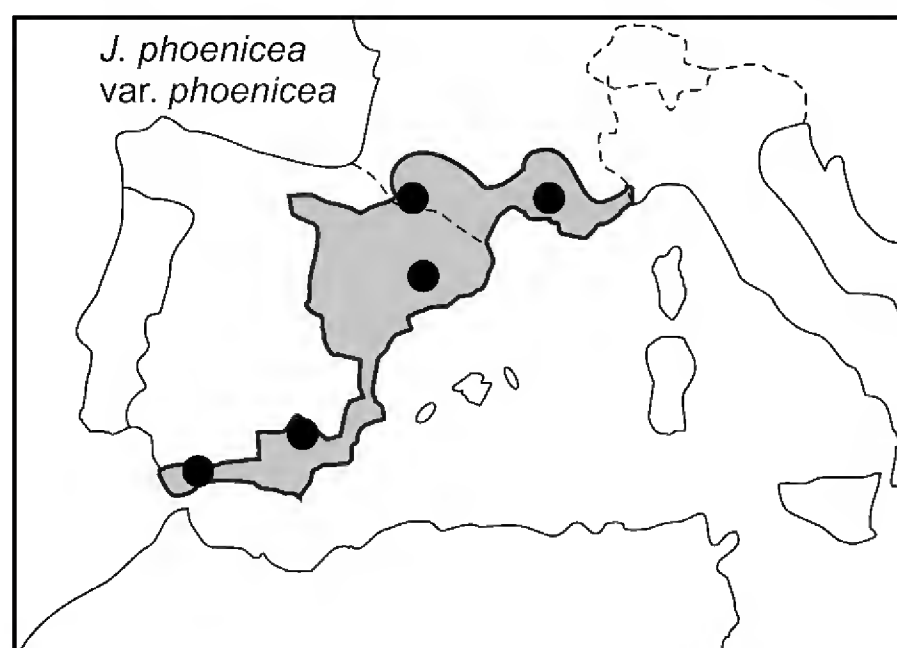


Figure 3. Distribution of *J. phoenicea* var. *phoenicea* showing populations sampled in this study (closed circles)

RESULTS AND DISCUSSION

Sequencing nrDNA and petN-psbM resulted in 2131 bp of data. The Bayesian tree shows (Fig. 4) all populations of *J. phoenicea* (var. *phoenicea*) in a well-supported clade, separated from *J. turbinata* (= *J. phoenicea* var. *turbinata*).

The El Penon population shows the largest differentiation (Fig. 4). France and Zaragoza populations show small differences (Fig. 4). No substitutional differences were found between the Andorra and Grazalema populations (Fig. 4).

To examine this geographical pattern, the order of linkage in the Bayesian tree (Fig. 4, *J. phoenicea* clade) was contour-mapped (Fig. 5). This shows the Andorra (AN) population linked to the Grazalema (GR) population (no substitutional differences found). Next, the Zaragoza (ZA) population enters the tree; then, the France (FA) population enters (Fig. 5). The most divergent population (El Penon, EP) enters the tree as the last member of the *J. phoenicea* clade (Fig. 5).

The uniformity of the *J. phoenicea* populations is shown in their petN-psbM sequences that had only one substitution (in the France population) and two indels. One 19 bp indel was present in both samples from Grazalema and one sample from El Penon. The other indel, a poly A 10-mer, was in all samples, except for an 11-mer in one sample from France and El Penon and found as a 12-mer in the second sample from France.

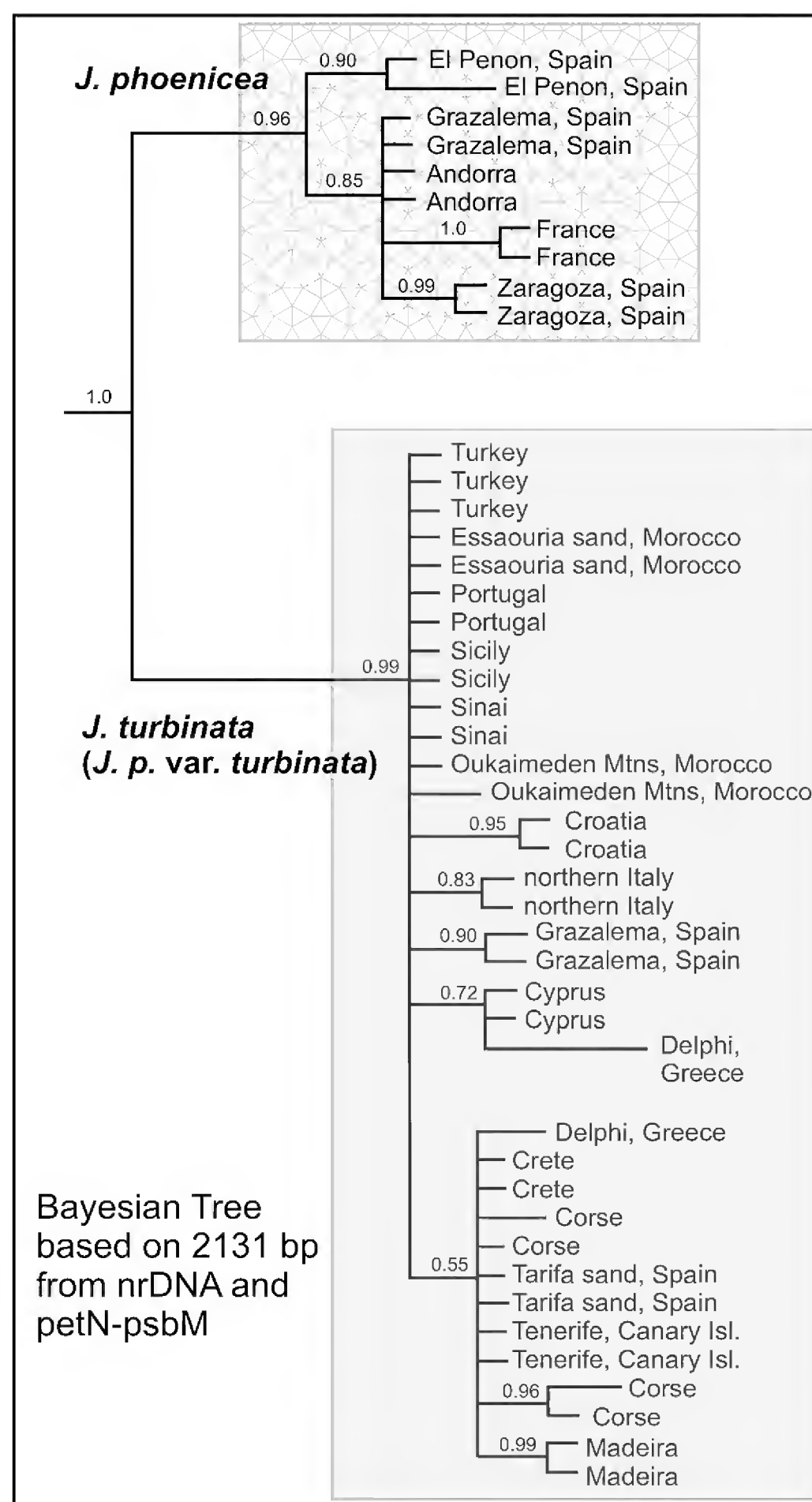


Figure 4. Bayesian tree for *J. phoenicea* populations (upper clade). *Juniperus turbinata* samples included for reference. Numbers at the branch points are posterior probabilities (1.0 - 0.0).

The *J. phoenicea* populations were a little less uniform in their nrDNA with 5 substitutions and one indel. One substitution event was found in both Zaragoza individuals. Another substitution was present in both El Penon samples and the third substitution event was present in both trees from France. The final two substitution events were present in only one tree: 7078 from El Penon. The 3-bp indel was absent in both trees from El Penon and one sample from Grazalema.

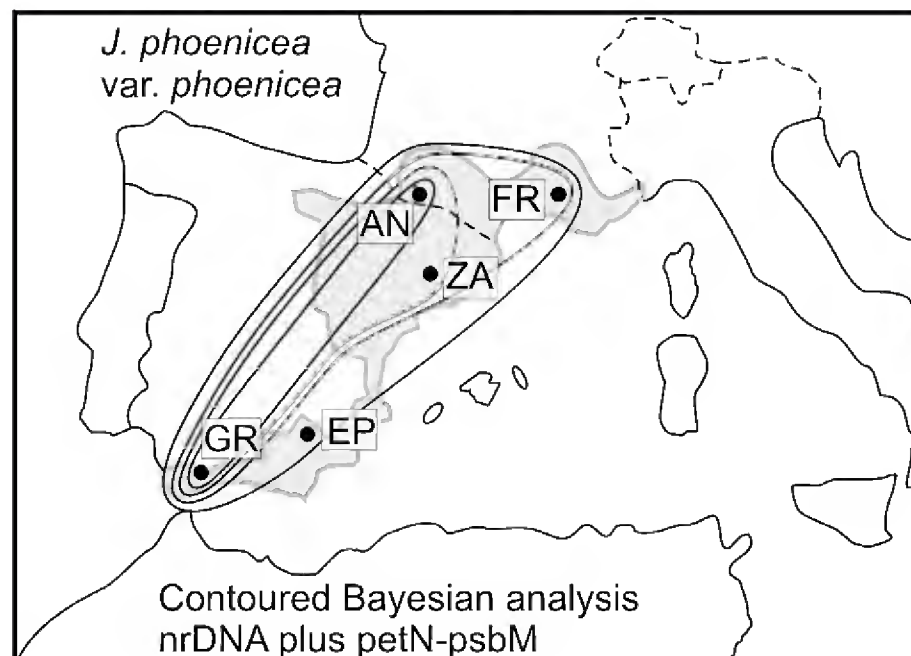


Figure 5. Contoured Bayesian analysis of *J. phoenicea* populations based on nrDNA and petN-psbM. AN = Andorra, FR = France, ZA = Zaragoza, GR = Grazalema, EP = El Penon.

In our study of geographic variation in the leaf essential oil from these same five populations of *J. phoenicea* (Adams et al., 2014), the leaf oils of one individual from Zaragoza and all the trees from Grazalema contained large amounts of typical heartwood oil components (α -cedrene, cis-thujopsene, α -alaskene, allo-cedrol, cedrol, etc.). When we corrected the leaf constituents (by removing the heartwood components and re-normalizing the percentages), there was little variation among the five populations, except for four compounds in the El Penon population: myrcene, β -phellandrene, α -terpineol were in higher concentrations, whereas, (E)-caryophyllene was present in a lower concentration. The divergence of the El Penon trees is congruent with the divergence in the DNA data presented in this study. Additional research is needed to understand the divergence of the El Penon population.

ACKNOWLEDGEMENTS

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Comparison of volatile leaf terpenoids from *Lippia dulcis* (Verbenaceae) obtained by steam distillation and pentane liquid extraction

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ABSTRACT

Comparison of pentane extraction and steam distillation of intact leaves of *Lippia dulcis* (hierbia dulce, ex Mexico) yielded 2.13% (DW basis) vs. 0.13% for pentane extraction (18 h, shaking). Analyses of the oils showed (pentane; distilled): camphor (33.9, 21.2%), camphene (1.8, 12.7%), 6-methyl-5-hepten-2-one (7.9, 3.4%), (+)-hernandulcin, the sweet sesquiterpene (9.2, 5.9%), 3-methyl-2-cyclohexene-1-one (2.7, 0.4%). The differences in hernandulcin by extraction method seems to be due to the different effects of pentane solvent on intact leaves (vs. steam volatilization by distillation). The pentane extract was also high in free, long chain fatty acids such as linoleic, hexadecanoic, and octadecanoic acids. Quantative data are presented for 72 components. Varying GC injector temperature from 100°C to 220°C revealed degradation of hernandulcin between 200°C and 220°C. Published on-line www.phytologia.org *Phytologia* 96(3): 252-259 (July 1, 2014). ISSN 030319430

KEY WORDS: *Lippia dulcis*, leaf terpenoids, hernandulcin, pentane extraction, steam distillation, degradation.

Lippia dulcis Trevir. (Verbenaceae) is a sweet-tasting, woody herb sold as hierbia dulce, hierbia buena, yerba dulce, Orozuz and other names in Mexico and central America (Compadre, Robbins and Kinghorn, 1986). The sweet taste is due to the presence of hernandulcin, a bisabolane-type sesquiterpene. It has been rated as 1000 times sweeter than sucrose (Compadre, Robbins and Kinghorn, 1986). The reader is referred to Compadre, Robbins and Kinghorn (1986) who give an excellent historical review of the folk-lore and traditional medicinal uses of the species.

The nomenclature has been subject to controversy. Moldenke (1934) considered *Lippia dulcis* as part of *Phyla* and renamed it *Phyla dulcis* (Trevir.) Moldenke, *Torreyia* 34: 9, 1934. Floras in Ecuador (Jorgensen and Leon-Yanez, 1999) and Nicaragua (Stevens, et al., 2001) continue to place it in *Phyla*. The Germplasm Resources Information Network (GRIN), USDA (<http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?429352>, search 20 Aug. 2014) currently recognizes *Phyla dulcis*. However, IPNI (International Plant Names Index, Kew) recognizes *Lippia dulcis*, with *Phyla dulcis* treated as a synonym (search 20 Aug. 2014). Marx et al. (2010), in a study of the phylogeny and classification of the Verbenaceae using seven cp DNA markers, found *Phyla* species to occur in a well-supported clade, four *Lippia* species in a clade with *Lantana*; *Lippia dulcis* occurred outside both the *Phyla* and *Lantana* clades. *Lippia* is a large genus of over 200 species, and their study only included six *Lippia* species. Nevertheless, because the traditional species of *Phyla* formed a well-supported clade, with the exclusion of *Lippia dulcis*, their study supports the concept that *Lippia dulcis* is not a part of *Phyla*.

More recently, O'Leary and Mulgura (2012), in their revision of the genus *Phyla*, explicitly excluded *Phyla dulcis* and *P. stochaedifolia* from the genus, stating "these are considered here to be better

placed under *Lippia*, given that both species lack malpighiaceous hairs, which are characteristic of the genus *Phyla* and are woody shrubs rather than the herbaceous habit noted for all *Phyla* species considered herein". In the present report, it is treated as *Lippia dulcis*.

Reports on the composition of the leaf terpenoids of *L. dulcis* have been variable (Table 1). Nayal et al. (2009) and Gornmann et al. (2008) reported 32.6% camphor and 10% hernandulcin from *L. dulcis* grown from seeds from M. P. Gupta, Panama (Table 1). However, the same laboratory (Nayal et al. 2009) reported 0.02% camphor and 14.5% hernandulcin from plants grown from Panama seeds (ex M. P. Gupta seed lot). It may be that the report by Gornmann et al. (2008), from that same laboratory, erroneously reported the composition of Mexico *L. dulcis* for their 'Panama' plants.

Kaneda et al. (1992) found no camphor but 0.154% hernandulcin in market plants from Valle de Anton, Cocola, Puerto Rico, sold for the treatment of respiratory ailments. This plant (identified as *L. dulcis*) was listed in the Flora of Panama as *Phyla scaberrima* (A. L. Juss.) Moldenke. Kaneda et al. (1992) also identified a new sweet sesquiterpene, (+)-4 β -hydroxyhernandulcin as well as (-) epi-hernandulcin from their Panama plants. Mori and Kato (1986) synthesized all four isomers of hernandulcin and noted that only the 6S, 1'S isomer (i.e., (+) hernandulcin) was sweet.

The presence of a large amount of camphor in the Mexican plants is of considerable interest because there are conflicting reports of none (or trace amounts of camphor in plants) from Panama, Puerto Rico and Columbia (Table 1). Because camphor is very heat-stable, it seems unlikely that the trace or absence of camphor in the Panama, Puerto Rico, Columbia and Brazil samples is due to decomposition; more likely, it is due to the lack of camphor in these plants. Although all the studies cite "plants identified by taxonomist," it is very possible that some of the samples may have been misidentified or there may be chemical races or chemotypes present in *L. dulcis* as suggested by Souto-Bachiller et al. (1997). Souto-Bachiller et al. (1997) concluded that 'tazonpelic xihuitl' ascribed to Francisco Hernandez by Aztec physicians more than 400 years ago (Anderson, 1977) is, in fact, 'yerba dulce' of Puerto Rico. Research on geographic variation in the leaf oils of *L. dulcis* is needed to clarify the problem.

Souto-Bachiller et al. (1996) collected seeds in 1990 from plants in Orocovis, Puerto Rico. They obtained high hernandulcin yields (18-26 mg/g), with no camphor from germinated shoots (6-8 weeks old). After repeated sub-culturing for five years, there were little effects on the oil composition, implying that the oils are genetically stable.

Oliveira et al. (2010) reported no camphor and 19.2% hernandulcin (Table 1) in plants grown in Brazil extracted by supercritical CO₂. Recently, Attia, Kim and Ro (2012) reported on molecular cloning of (+)-epi- β -bisabolol synthase as a precursor to the biosynthesis of hernandulcin.

Compardre et al. (1986) discovered that hernandulcin decomposes upon heating to 140°C. They tried to compensate for this problem by running their GC injector at 70°C, but this is too low to quantitatively transfer a board mixture of volatile components to the GC column. Souto-Bachiller et al. (1997) found a solution was to run a narrow bore injector liner (0.75 mm bore) so that the dead volume is small and the sample quickly transferred from their injector (220°C) to the cool (60°C) column. Even using this method, they appeared to have decomposition of hernandulcin, as indicated by the presence of 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohexen-1-one (putative decomposition products of hernandulcin). Souto-Bachiller et al. (1997) extracted with pentane and dichloromethane (sequentially with combined extracts) because they thought that distillation would lead to decomposition. It may be that they were considering water-distillation where the plants are placed in water and boiled to co-produce steam and volatile oil. Water-distillation (or hydro-distillation) is well known to produce artifacts due action of acids from the leaching of organic leaf acids into the water (Adams, 1991). A safer steam

distillation can be performed in all glass units, with the plant materials suspended about boiling water, so that the oil is not exposed to leached-out organic acids. As the maximum temperature reached is 100°C and contact is only with glass, this type of steam distillation eliminates decomposition for all but the most labile components in nature. See Adams (1991) for a diagram of this type of steam distillation apparatus.

Table 1. Reports on the amounts of camphor and hernandulcin in *Lippia dulcis*.

publication	plant source	camphor %	hernandulcin %	extraction
Compadre et al. 1986	Mexico (local markets) ¹	53.2	0.004 [#]	steam distilled, 2h
Nayal et al. 2009	Mexico (Helenion Nursery, Germany) ²	32.6	10.1	distilled, 4h
Gornmann et al. 2008	Panama (seeds, M. P. Gupta, Panama) ³	32.6	10.0	steam distilled, 4h
Nayal et al. 2009	Panama (seeds, M. P. Gupta, Panama) ²	0.02	14.5	distilled, 4h
Kaneda et al. 1992	Puerto Rico (market, Valle de Anton) ¹	none	0.154	petroleum ether
Souto-Bachiller et al. 1997	Puerto Rico (plants, ex Orocovis) ¹	<0.01	22.0	pentane & CH ₂ Cl ₂
Moreno-Murillo et al. 2010	Colombia (plants, ex Tenza Valley) ⁴	none	1.1*	hydro-distillation, 3h
Oliveira, et al. 2010.	Brazil(local plants?) ¹	none	19.2	supercritical CO ₂
Present study	Mexico (seeds, Chiltern Seeds, UK) ⁵	21.2	9.2	pentane overnight
Present study	Mexico (seeds, Chiltern Seeds, UK) ⁵	33.9	4.5	steam distilled, 4h

¹dried and milled; ²air dried, 30°C and cut; ³dried and cut; ⁴fresh or dried?; ⁵fresh leaves.

*(ca. 4-5%, hernandulcin was mostly decomposed during GC analysis)

[#]severely decomposed during GC analysis.

Table 2. Comparison of column conditions vs. hernandulcin yields.

source	publication	hernandulcin %	column	injector	init. col. temp	split	liner bore
Mex.	Compadre et al. 1986	0.004 [#]	DB-1	70°C	35°C	1/17	unknown
Mex.	Nayal et al. 2009	10.1	HP-5	220°C	60°C, 30 sec.	none	0.75 mm bore
Pan.	Gornmann et al. 2008	10.0	HP-5	220°C	60°C, 30 sec.	none	0.75 mm bore
Pan.	Nayal et al. 2009	14.5	HP-5	220°C	60°C, 30 sec.	none	0.75 mm bore
PR	Kaneda et al. 1992	0.154	silica gel chromatography, id by NMR (no GC/MS)				
PR	Souto-Bachiller et al. 1997	22.0	SPB-5	220°C	60°C, 30 sec.	none	0.75 mm bore
Col.	Moreno-Murillo et al. 2010	1.1*	HP-5	200°C	70°C, 2 min.	1/10	0.75 mm bore
Bra.	Oliveira, et al. 2010	19.2	OV-5	250°C	50°C	?	?
Mex.	Present study	9.2	DB-5	220°C	60°C	1/10	4.0 mm bore ^{\$}

^{\$}packed with deactivated, silane treated glass wool

The purpose of this study was to compare the effects of pentane extraction and steam distillation on the composition of the volatile leaf oil of Mexican *Lippia dulcis* and to examine the effects of variation in injection temperatures on the degradation of hernandulcin.

MATERIALS AND METHODS

Plant material: *Lippia dulcis* seeds were obtained from Chiltern Seeds, UK, via M. Attia, University of Calgary, Canada and grown in the greenhouse. Vegetatively propagated plants were grown under partial shade in pots or in the field of the experimental farm at Prairie View A&M University. Fresh leaves were collected from young plants.

Essential oils analysis - Set: A: 10 g FW (2.2 g DW) of fresh leaves, plus 20 ng of methyl decanoate (Internal Standard) was extracted with pentane on a rotary shaker for 18 h. Set B: 30.1 g FW (4.79 g DW) of fresh, greenhouse, mature leaves with 2 mg of methyl decanoate added (as an internal standard) steam distilled for 4 h using a modified circulatory Clevenger-type apparatus (Adams 1991). Extracts were concentrated (pentane or diethyl ether trap-removed) with nitrogen and stored at -20°C until

analyzed. Extracted leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt. / (oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 µl of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 220°C, detector 240°C, scan time 1/sec, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

RESULTS AND DISCUSSION

The pentane extraction (18 h) yielded 0.13% (W/W) oil, compared to 2.13% for steam distillation of 4h (Table 3). Examination of the extracted leaves after drying, revealed that the pentane-extracted leaves still had the typical sweet, camphor aroma, whereas the steam-distilled leaves were without odor. It is clear that the pentane extraction of intact leaves was not very complete. The leaves were not ground in this study to minimize the possibility of enzymatic and/ or free radical degradation of components. It is very probable that grinding the leaves would have increased the pentane extractables yields.

In addition to yields, the oils were very different in composition. The pentane extract contained 4.7 and 3.4% of the very volatile (E)-ε-hexenal and (Z)-3-hexenol, versus trace in the steam distilled oil. It is common in steam distillation to lose some of the very volatile compounds. The yields of hernandulcin and epi-hernandulcin in the pentane extract was higher (9.2, 2.7%) than in the steam distilled oil (4.5, 0.7%). However, this may be due lower efficiency of pentane solvent than volatility (steam distillation) for many compounds (cf. pentane vs steam): camphene - 1.8, 12.7%; limonene - 0.7, 4.6%; camphor - 21.2, 33.9%; α-copaene - 1.5, 4.0%; (E)-caryophyllene - 2.2, 6.0%; (E)-β-farnesene - 0.8, 2.3%.

In contrast, some components were larger in the pentane extract (pentane, steam): 1-octen-3-ol - 4.1, trace; 6-methyl-5-hepten-2-one - 7.9, 3.9; benzene aldehyde - 1.8, 0.0; 3-methyl-2-cyclohexene-1-one - 4.7, 0.7; phenyl ethyl alcohol - 4.0, 0.0. The much larger amount of 1-octen-3-ol may be due to the co-solvent effects of alkanes. Phenyl ethyl alcohol may be so labile that it is degraded in the steam distilled oil.

In addition to the volatile terpenoids, large amounts of hexadecanoic, octadecanoic, and linoleic acids were removed by the pentane extractions, along with minor amounts of long chain alkanes (associated with waxy cuticles). These compounds were absent or minor in the steam distilled oil (Table 3).

The work by Kaneda et al. (1992) is relevant in that they used traditional silica gel chromatography to separate and identify (by NMR) hernandulcin, epi-hernandulcin, and 6-methyl-5-heptene-2-one from *Lippia dulcis* from Panama. Although silica gel chromatography/ NMR is a slower procedure than GC/MS, no high temperatures are encountered, so degradation hernandulcin should not occur. They reported 6-methyl-5-hepten-2-one, 0.043% (w/w); hernandulcin, 0.154%(w/w). Since none of the 6-methyl-5-hepten-2-one should have arisen by degradation of hernandulcin, it follows that 6-methyl-5-hepten-2-one is a naturally occurring component of *Lippia dulcis* oil.

The ratio of 6-methyl-5-hepten-1-one / hernandulcin may be an indicator of the severity of degradation of hernandulcin. The degradation ratio was essentially equal for pentane (0.86) and steam distillation (0.87), indicating that the same amount of degradation occurred in each extraction. Because of

the low temperature during pentane extraction, it seems unlikely the hernandulcin degraded during the extraction phase, but rather in GC analysis phase. Because their degradation ratios are the same, it appears that no degradation of hernandulcin occurred during steam distillation. So either pentane or steam distillation could be used to extract *L. dulcis* oils (but a study should use only one method).

To further examine the effects of inlet injector temperature on the degradation of hernandulcin, a series of analyses were made, using one 4h distilled oil sample, by increasing the injector temperature (Table 4). Hernandulcin content was lowest at 100°C, then increased to 160°C, then declined from 200°C to 220°C (Fig. 1). It seems likely that the high variance at 100°C and lower amount of the less volatile sesquiterpene, hernandulcin, is due to incomplete volatilization in the injector and selective loss of hernandulcin in the split line. The decline at 220°C is due to decomposition of hernandulcin (Fig. 1). The rather constant nature of 6-methyl-5-hepten-2-one (Fig. 1), followed by the sudden increase at 220°C, seems to indicate that most 6-methyl-5-hepten-2-one is a natural product in the oil and only a small portion was derived from the decomposition of hernandulcin (220°C, Fig. 1). Alternatively, there could have been some decomposition of hernandulcin during harvest, storage and/ or extraction.

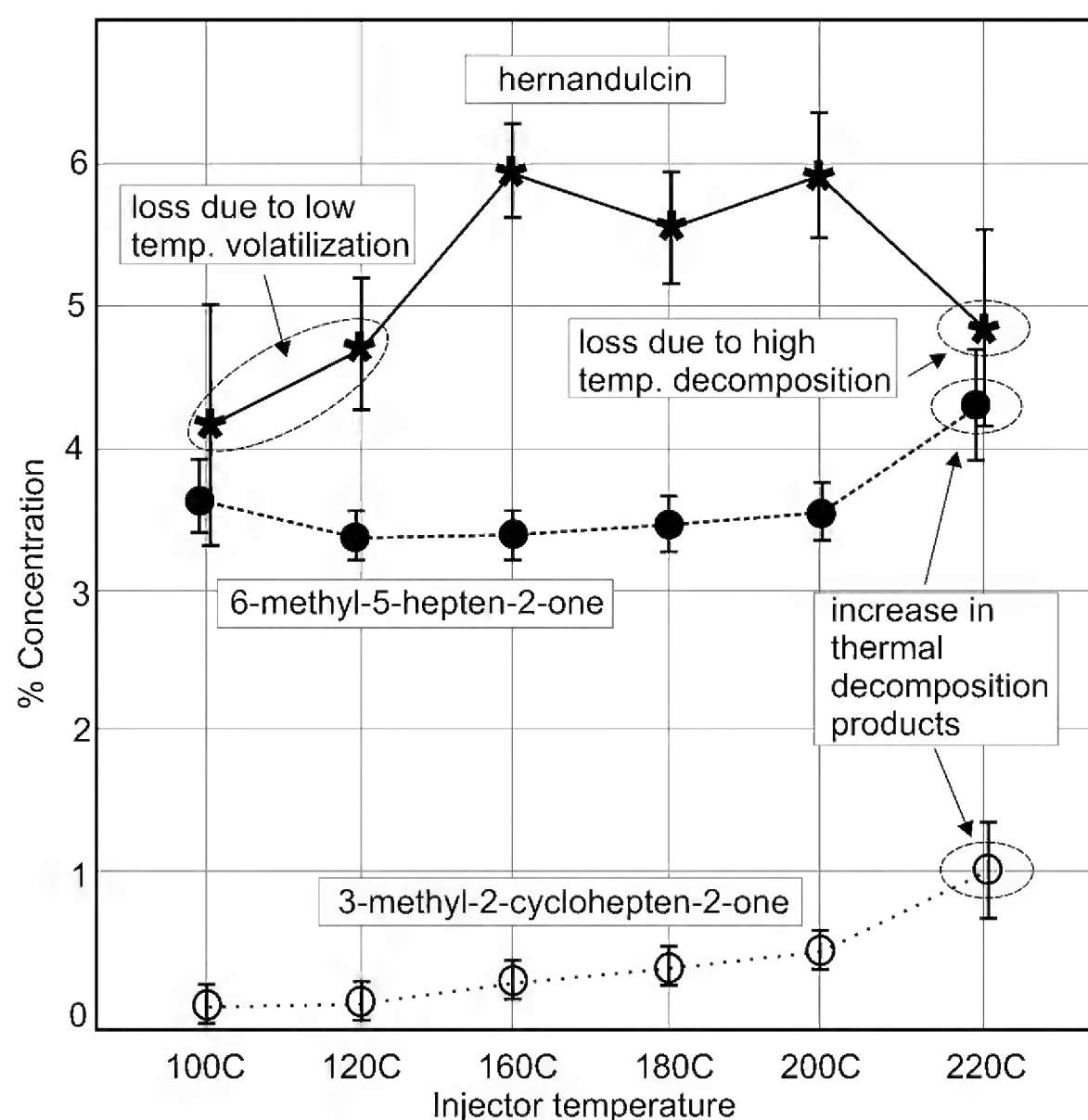


Figure 1. Plots of hernandulcin versus putative decomposition products: 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohepten-2-one with changes in the injector temperature for GC analyses.

The concentration of 3-methyl-2-cyclohepten-2-one was very stable from 100°C to 200°C, then increased at 220°C (Fig. 1, Table 4). This suggests that the increase at 220°C is due to the decomposition of hernandulcin. Small amounts of 3-methyl-2-cyclohepten-2-one may be naturally present in the oil.

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Table 3. Comparison oil compositions of *Lippia dulcis* (Mexican *Lippia* or Orozuz) extracted in pentane (RT, overnight) vs. steam distilled (4h). t < 0.1%, NI = not integrated. GC injector run at 220°C.

KI	Compound	pentane ext.	steam distilled
	percent oil yield (DM basis)	0.13%	2.13%
846	(E)-2-hexenal	4.7	t
850	(Z)-3-hexenol	3.4	t
921	tricyclene	-	0.1
932	α -pinene	0.2	2.1
946	camphene	1.8	12.7
952	benzyl aldehyde	0.1	-
974	1-octen-3-ol	4.1	t
974	β -pinene	t	0.6
981	6-methyl-5-hepten-2-one	7.9	3.4
988	myrcene	0.2	1.4
1024	limonene	0.7	4.6
1026	benzyl alcohol	t	-
1036	benzene acetaldehyde	1.8	-
1046	3-me-2-cyclohexene-1-one	4.7	0.4
1086	terpinolene	0.3	1.7
1095	linalool	t	0.4
1100	n-nonanal	t	-
1106	phenyl ethyl alcohol	4.0	-
1141	camphor	21.2	33.9
1150	(2E,6Z)-nonadienal	0.3	-
1165	borneol	0.4	0.5
1179	p-cymen-8-ol	0.2	0.1
1186	α -terpineol	0.5	0.1
1190	methyl salicylate	t	-
1345	α -cubebene	-	0.1
1356	eugenol	0.5	-
1374	α -copaene	1.5	4.0
1387	β -bourbonene	0.1	0.5
1387	β -cubebene	-	0.1
1409	α -gurjunene	-	0.1
1417	(E)-caryophyllene	2.2	6.0
1432	α -trans-bergamotene	-	0.3
1440	(Z)- β -farnesene	-	0.2
1452	α -humulene	-	0.3
1454	(E)- β -farnesene	0.8	2.3
1464	9-epi-(E)-caryophyllene	-	0.3
1469	dehydro-sesquicineole	-	0.3
1478	γ -muurolene	0.2	0.3
1480	germacrene D	0.3	1.1
1500	α -muurolene	0.4	0.6
1505	β -bisabolene	0.3	1.0
1514	cubebol	-	0.1
1522	δ -cadinene	2.2	3.8
1561	(E)-nerolidol	0.2	0.3
1582	caryophyllene oxide	1.3	0.6
1622	sesquiterp., 85,93,136,218	-	0.7
1630	muurola-4,10(14)-dien-1- β -ol	0.4	0.2
1639	caryophylla-4(12),8(13)-dien-5- β -ol	-	0.1
1644	α -muurolol	-	t
1660	cis-calamen-10-ol	0.3	-
1662	sesquiterp., 43,109,218,236	-	1.7
1667	isomer of 1662	-	1.0
1683	α -bisabolol	0.1	0.1

1685	epi- α -bisabolol	2.6	2.6
1722	methyl-tetradecanoate	1.4	-
1764	sesquiterp., <u>111</u> ,55,178,228	0.9	-
1783	sesquiterp., <u>43</u> ,111,135,236	1.7	-
1849	sesquiterp., <u>175</u> ,189,217,232	-	-
1851	(+) hernandulcin	9.2	5.9
1865	(-) epi-hernandulcin	2.7	0.7
1892	triene, hydrocarbon, <u>79</u> ,67, 93,248	1.3	-
1921	methyl-hexadecanoate	1.0	-
1933	cyclohexadecanolide	1.0	-
1959	hexadecanoic acid	10.7	1.2
2113	hydrocarbon, <u>71</u> ,123,55,296	0.6	0.8
2124	methyl octadecanoate	0.6	-
2132	linoleic acid	35.5	2.5
2158	octadecanoic acid	14.2	1.0
2200	docosane	1.1	t
2300	tricosane	0.5	t
2400	tetracosane	0.4	0.9
2500	pentacosane	0.2	0.1

KI = linear Kovats Index on DB-5, 30m column.

Table 4. ANOVA for selected components of *Lippia dulcis* oil distilled 4h and injected at 100°C, 120°C, 160°C, 180°C, 200°C and 220°C. F ratio from ANOVA, Signif.: *** = P=0.001; ** =P = 0.05. nt = not tested by ANOVA.

KI	Compound	100°C	120°C	160°C	180°C	200°C	220°C	F ratio	Signif.
981	6-methyl-5-hepten-2-one	3.66	3.38	3.38	3.47	3.55	4.29	5.52	***
1046	3-me-2-cyclohexene-1-one	0.10	0.10	0.30	0.38	0.50	1.02	18.19	***
1851	(+) hernandulcin	4.18	4.78	5.86	5.51	5.85	4.79	5.22	*
1865	(-) epi-hernandulcin	0.59	0.47	0.41	0.36	0.43	0.68	nt	nt

The leaf essential oils of *Picea chihuahuana* Martinez and *P. martinezii* Patterson (Pinaceae)**Robert P. Adams**

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ABSTRACT

The first comprehensive analysis of the volatile leaf terpenoids of *Picea chihuahuana* and *P. martinezii* is reported. The volatile leaf oil of *P. chihuahuana* is dominated by bornyl acetate (47.9%) with moderate amounts of α -pinene (4.7), camphene (6.3), β -pinene (5.2), camphene (6.2), limonene (7.4) and β -phellandrene (7.3%). The leaf oil of *P. martinezii* is dominated by bornyl acetate (26.4%), α -pinene (16.6) and myrcene (15.1%), with moderate amounts of camphene (5.6) and β -pinene (9.7%). For such closely related species (Patterson and Harrod, 1994), the oils are quite differentiated. Components unique in *P. chihuahuana* oil are: α -phellandrene, cis- and trans-p-menth-2-en-1-ol, cis- and trans-piperitol, piperitone, cis- and trans-piperitol acetate, methyl isopimarate, methyl levopimarate and methyl abietate. Unique compounds in *P. martinezii* oil include: α -muurolene, δ -cadinene, caryophyllene oxide, humulene epoxide II, epi- α -cadinol, epi- α -muurolol, α -muurolol, α -cadinol, germacrene-4(15),5,10(14)-triene-1-al and neo-abienal. Published on-line www.phytologia.org *Phytologia* 96(4): 260-263 (Oct 1, 2014). ISSN 030319430

KEY WORDS: *Picea chihuahuana*, *P. martinezii*, terpene composition, volatile leaf oils, Pinaceae.

Taylor, Patterson and Harrod (1994) used a combination of morphology, phenolics and terpenoids to examine Mexican species of *Picea*. Of interest to the present study, was the observation that *Picea chihuahuana* and *P. martinezii* were shown to be distinct species by their morphology (Fig. 2), phenolics (Fig. 5) and terpenoids (Fig. 6), re-confirming their previous study (Taylor and Patterson, 1980). Taylor, Patterson and Harrod (1994) listed α -pinene, camphene, β -pinene, myrcene, γ -terpinene, piperitone, citronellol, geraniol, camphor, and bornyl acetate as present in six species of *Picea* examined, but did not give any composition data.

Recently, Jaramillo-Correa et al. (2006) examined mt and cp DNA markers among *P. chihuahuana* and *P. martinezii* and concluded that they are not conspecific, but distinct species.

Aside from the qualitative report of Taylor, Patterson and Harrod (1994), no reports have been published on the composition of the leaf volatile oils of *Picea chihuahuana* or *P. martinezii*. The purpose of the present paper is to give the first detailed report on the composition of the volatile oils of these species.

MATERIALS AND METHODS

Leaf samples were collected from *Picea chihuahuana* (Adams 14329-14331) from trees cultivated at Medford city park, Medford, OR (grown from seed collected on Rio Oteros, near Creel, Chihuahua, Mexico) and from *P. martinezii* (Adams 14326-14328) cultivated at Frank Callahan's land, Central Point, OR, grown from seed collected by Frank Callahan in Nuevo Leon, Mexico. Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The leaf oils of *P. chihuahuana* and *P. martinezii* were clear in color and the yields (w/DW) very small: 0.34% and 0.25%, respectively. The volatile leaf oil of *P. chihuahuana* is dominated by bornyl acetate (47.9%, Table 1) with moderate amounts of α -pinene (4.7), camphene (6.3), β -pinene (5.2), camphene (6.2), limonene (7.4) and β -phellandrene (7.3%). The leaf oil of *P. martinezii* is dominated by bornyl acetate (26.4%, Table 1), α -pinene (16.6) and myrcene (15.1%) with moderate amounts of camphene (5.6) and β -pinene (9.7%). For such closely related species (Patterson and Harrod, 1994), the oils are quite different (Table 1). There are several unique oil components in *P. chihuahuana*: α -phellandrene, cis- and trans-p-menth-2-en-1-ol, cis- and trans-piperitol, piperitone, cis- and trans-piperitol acetate, methyl isopimarate, methyl levopimarate and methyl abietate. Unique oil compounds in *P. martinezii* include: α -muurolene, δ -cadinene, caryophyllene oxide, humulene epoxide II, epi- α -cadinol, epi- α -muurolol, α -muurolol, α -cadinol, germacra-4(15),5,10(14)-triene-1-al and neo-abienal (Table 1).

The leaf volatile terpenoids data support the conclusions of Patterson and Harrod (1994) and Jaramillo-Correa et al. (2006) that *P. chihuahuana* and *P. martinezii* are distinct species.

It might be noted that *P. chihuahuana*, in cultivation in Medford, OR (Fig. 1), is a robust, fast growing tree that will likely be widely cultivated for its glaucous foliage, shape and hardiness.

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Figure 1. *Picea chihuahuana*, seed cone (insert) in cultivation at the Medford City Park, OR with Frank Callahan.

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Table 1. Comparison of leaf oil compositions of *Picea chihuahuana* and *P. martinezii*. Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column.

KI	compound	<i>chihuahuana</i>	<i>martinezii</i>
846	(E)-hexenal	0.2	0.3
921	tricyclene	0.4	0.4
932	α-pinene	4.7	16.6
946	camphene	6.3	5.6
969	sabinene	t	t
974	β-pinene	5.2	9.7
988	myrcene	3.2	15.1
997	ethyl hexanoate	0.2	-
1002	α-phellandrene	0.7	-
1008	δ -3-carene	t	-
1014	α -terpinene	0.1	-
1020	p-cymene	t	t
1024	limonene	7.4	1.6
1025	β-phellandrene	7.3	2.4
1032	(Z)- β -ocimene	t	-
1054	γ -terpinene	t	t
1086	terpinolene	1.2	1.2
1095	linalool	0.3	0.2
1118	cis-p-menth-2-en-1-ol	0.9	-
1122	α -campholenal	0.1	0.3
1136	trans-p-menth-2-en-1-ol	0.7	-
1136	trans-pinocarveol	-	0.1
1141	camphor	t	0.1
1145	camphene hydrate	0.5	0.2
1165	borneol	1.7	0.5
1174	terpinen-4-ol	0.1	0.2
1186	α -terpineol	1.1	1.1
1190	methyl salicylate	-	t
1195	cis-piperitol	0.3	-
KI	compound	<i>chihuahuana</i>	<i>martinezii</i>
1207	trans-piperitol	0.6	-

KI	compound	<i>chihuahuana</i>	<i>martinezii</i>
1218	endo-fenchyl acetate	t	-
1223	citronellol	t	-
1249	piperitone	0.6	-
1287	bornyl acetate	47.9	26.4
1298	trans-pinocarvyl acetate	0.4	0.3
1324	myrtenyl acetate	0.1	-
1332	cis-piperitol acetate	0.1	-
1343	trans-piperitol acetate	0.2	-
1350	α -longipinene	0.1	-
1379	geranyl acetate	-	0.1
1396	duvalene acetate	t	0.1
1417	(E)-caryophyllene	0.3	2.1
1454	(E)- β -farnesene	t	-
1469	n-dodecanol	t	-
1480	germacrene D	t	1.1
1500	α-muurolene	-	0.5
1522	δ-cadinene	-	0.2
1565	dodecanoic acid	t	-
1582	caryophyllene oxide	-	0.3
1608	humulene epoxide II	-	0.3
1638	epi-α-cadinol	-	0.2
1640	epi-α-muurolol	-	0.2
1644	α-muurolol	-	0.1
1652	α-cadinol	-	0.8
1685	germacra-4(15),5,10(14)-triene-1-al	-	0.3
1959	hexadecanoic acid	0.2	0.5
1987	iso-pimara-7,15-diene	0.3	0.2
2182	hexadecanoic acid, butyl ester	t	-
2222	abietal isomer	0.3	0.9
2298	methyl isopimarate	0.2	-
2300	tricosane	0.2	0.4
2306	methyl levopimarate	0.4	-
2313	abietal	0.5	0.6
2375	neo-abienal?	-	0.4
2385	methyl abietate	0.1	-